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## Human antithrombin III mutants.

A novel human antithrombin III (AT III) mutant having a high antithrombin activity in the absence of heparin deffective in the treatment of thrombotic disorders as an anticoagulant, which is obtained by mutating amino do at the reactive site and the heparin binding site of human AT III into another amino acids with the use of recombinant DNA technology with the use of a DNA coding for AT III as a template.

A method for mass producing the above-described mutant by incubating a host transformed by an pression vector having the cDNA of the mutant inserted therein.

### Background of the Invention

Field of the Invention

The present invention relates to a human antithrombin III (AT III) mutants which are obtained by mutating one or more amino acid(s) in the amino acid sequence of human AT III into another amino acid(s) and exhibit high antiprotease activities even in the absence of heparin. These human AT III mutants are usable as a remedy for thrombotic disorders.

#### Description of the Related Art

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Anticoagulant activity of glycosaminoglycans including heparin is mediated by antithrombin III (AT III) and heparin cofactor II (HC II) contained in the blood. AT III and HC II are serine protease inhibitors which are called serpins in general. There has been often reported with respect to AT III among these substances that a decrease in the blood AT III level due to a congenital or acquired factor would result in thrombotic disorders. Accordingly, AT III plays a physiologically important role as a factor regulating the blood coagulation system consisting of a series of serine proteases.

It is known that human AT III is a glycoprotein of a molecular weight of approximately 60 kd which is mainly synthesized in the liver and contained in normal plasma at a concentration of about 150 µg/ml and that human AT III inhibits serine proteases participating in coagulation and fibrinolysis systems including thrombin and factor Xa. The primary structure of human AT III has been clarified by the direct determination of its amino acid sequence (see Petersen, T.E. et al., The Physiological Inhibitors of Blood Coagulation and Fibrinolysis, Elsevier Science Publishers, Amsterdam, 43, 1979) and cDNA cloning [see Bock, S.C. et al., Nucl. Acids Res., 10, 8113 (1982); Prochownik, E.V. et al., J. Biol. Chem., 258, 8389 (1982); Chandra, T. et al., Proc. Natl. Acad. Sci. USA, 80, 1845 (1983)]. According to these reports, human AT III is a single-chain glycoprotein consisting of 432 amino acids which is secreted and formed by excising a signal peptide of 32 residues from a precursor protein. It has four N-linked glycosylation sites in the molecule. The carbohydrate content is about 15% of the molecular weight.

Human AT III reacts with a serine protease such as thrombin at a ratio of 1:1 and thus forms a stable complex, thus inhibiting the activity of the protease. It is thought that, in this reaction, a peptide bond between the 393rd Arg residue and the 394th Ser residue in the molecule of human AT III is cleaved by the protease and an acyl bond is formed between the terminal Arg residue newly formed and the Ser residue at the active center of the protease. This Arg (393)-Ser (394) sequence is generally referred to as a reactive site.

The protease inhibition by AT III would relatively slowly proceed. When the reaction system contains heparin, however, the reaction is dramatically accelerated. Namely, the addition of heparin elevates the thrombin inhibition rate of AT III by more than 1,000 times. It is thought that this function mechanism proceeds as follows. When heparin binds to a specified site (heparin binding site) in AT III, the higher-order structure of AT III turns into a structure liable to undergo interaction with the protease. At the same time, the protease binds to the heparin molecule. Thus a ternary complex is apt to be formed. Further, from the physiological viewpoint, it is considered that heparin-like substances existing on the surface of vascular endothelial cells exert similar actions and thus play an important role in the mechanism for regulating the blood coagulation system by AT III.

There have been used so-called anticoagulants for treating and preventing thrombotic disorders induced by various causes. Heparin is one of highly important anticoagulants at present. However, it is reported that serious side effects are sometimes induced by the administration of heparin [see Amerena, J. et al., Adverse Drug React. Acute Poisoning Rev., 9, 1 (1990); Levine, M.N. et al., Semi. in Thrombos. Hemostas., 12, 39 (1986); Kelton, J.G. et al., ibid., 12, 59 (1986); Levine, M.N., ibid., 12, 63 (1986)]. Typical examples of these side effects include hemorrhage, thrombocytopenia, hypoadrenalism, hypersensitiveness, necrosis of the administration site and osteoporosis. When there is a high risk of hemorrhage in the fields of, for example, obstetrics and gynecology or postoperative treatments or in the case of a prolonged administration, heparin should be carefully used. Furthermore, it is reported that heparin promotes inactivation of AT III by elastase of neutrophils in vitro [see Jordan, R.E. et al, Science, 237, 777 (1987); Jordan, R.E. et al., J. Biol. Chem., 264, 10493 (1989)]. Thus care should be taken in the administration of heparin when elastase of neutrophils seemingly relates to the conditions of diseases such as serious infection or septicemia. In addition, the anticoagulant effect of heparin is essentially mediated by AT III and, therefore, can be scarcely expected in the case where blood AT III level is lowered.

Meanwhile, human AT III has been clinically applied to thrombophilia based on congenital AT III deficiency and disseminated intravascular coagulation syndrome (DIC) accompanied by a decrease in AT III in the form of a plasma derived AT III concentrate. As described above, however, AT III exhibits only a slow progressive antithrombin activity in the absence of heparin. Therefore the use of AT III alone is rather a supplementary treatment and its usefulness as an anticoagulant is limited. Thus attempts have been made to use AT III together with heparin or to prepare and use an AT III/heparin complex to thereby improve the usefulness of AT III as an anticoagulant. However, it is obvious that the above-mentioned disadvantages of heparin cannot be overcome even by these methods.

As described above, AT III has two functional sites, namely, the reactive site and the heparin-binding site. A number of reports have revealed that the amino acid sequence around the reactive site carries an important role in the expression of the function as a protease inhibitor as well as in the determination of inhibition specificity against various proteases. In congenital AT III anomaly such as AT III Hamilton wherein Ala at the 382-position has mutated into Thr [see Devraj-Kizuk, R. et al., Blood, 72, 1518 (1988)], AT III Cambridge I wherein Ala at the 384-position has mutated into Pro [see Perry, P.J. et al., FEBS Lett., 254, 174 (1989)], AT III Glasgow wherein Arg at the 393-position has mutated into His [see Erdjument, H. et al., J. Biol. Chem., 263, 5589 (1988)], AT III Pescara wherein Arg at the 393-position has mutated into Pro [see Lane, D.A. et al., J. Biol. Chem., 264, 10200 (1989)] and AT III Denver wherein Ser at the 394-position has mutated into Leu [see Stephens, A.W. et al., J. Biol. Chem., 262, 1044 (1987)], abnormal AT III molecules each has lost antiprotease activity and patients of these anomalies suffer from thrombotic disorders.

On the other hand, studies on congenital AT III molecule anomaly and results of chemical modification of amino acid residues have revealed amino acids directly relating to the heparin-binding site, namely, binding to heparin. Regarding the molecular anomaly, there have been reported AT III Rouen III wherein IIe at the 7-position has mutated into Asn [see Brennan, S.O. et al., FEBS Lett., 237, 118 (1988)], AT III-Rouen IV wherein Arg at the 24-position has mutated into Cys [see Borg, J.Y. et al., FEBS Lett., 266, 163 (1990)], AT III Basel wherein Pro at the 41-position has mutated into Leu [see Chang, J.Y. and Tran, T.H., J., Biol. Chem., 261, 1174 (1986)], AT III Toyama wherein Arg at the 47-position has mutated into Cys [see Koide, T. et al., Proc. Natl. Acad. Sci. USA, 81, 289 (1984)] and AT III Geneva wherein Arg at the 129-position has mutated into Gln [see Gandrille, S. et al., J. Biol. Chem., 265, 18997 (1990)]. Each of these abnormal AT IIIs has a lowered heparin affinity and cannot exert normal physiological functions, thus causing thrombotic diorders. Further, the results of experiments on chemical modification of amino acids suggest that amino acids including Trp at the 49-position, Lys at the 114-position, Lys at the 125-position, Arg at the 129-position, Lys at the 136-position and Arg at the 145-position might directly relate to binding to heparin [see Blackburn, M.N. et al., J. Biol. Chem., 259, 939 (1984); Peterson, C. et al., J. Biol. Chem., 262, 8061 (1987); Sun. X.J. and Chang, J.Y., Biochemistry, 29, 8957 (1990)].

Based on these findings, attempts have been made to improve AT III through substitution of an amino acid(s) of AT III. For example, ZettlemeissI et al. have disclosed a method for producing an AT III mutant having improved properties relating to heparin binding/heparin activation by mutating an amino acid(s) at the glycosylation site in AT III and another method for producing an AT III mutant having modified enzyme specificities by mutating an amino acid(s) at the reactive site (European Patent Publication-A No. 384122). Further, Dijkema et al. has reported a method for producing an AT III mutant having a modified antithrombin/antiXa activity by mutating an amino acid(s) at the reactive site (International Publication No. WO 91/00291).

However there has not been found out any human AT III mutant which is satisfactory from the clinical viewpoint. It is, therefore, urgently required to construct a human AT III mutant having an elevated activity of inhibiting thrombin or factor Xa in the absence of heparin.

It is an object of the present invention to provide novel human AT III mutants having a high antithrombin activity even in the absence of heparin. It is another object of the present invention to provide a method for mass producing said human AT III mutants by the recombinant DNA technology.

Disclosure of the Invention

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Summary of the Invention

At present, it is thought that the mechanism of enchancing the antiprotease activity of AT III by heparin would proceed as follows. First, heparin binds to the heparin binding site of AT III to thereby change the conformation of AT III into another one which can more easily react with a protease. At the same time, the protease binds to the same heparin molecule at the above-mentioned heparin binding site, thus elevating the rate of the formation of an AT III/protease complex [see Pletcher, C.H. and Nelsestuen, G.L., J. Biol.

Chem., 258, 1086 (1988)]. According to this hypothesis, the change in the configuration at the reactive site induced by the heparin binding to the heparin binding site of AT III is thought to be important in the enhancement of the antiprotease activity. This fact suggests that an AT III mutant exhibiting an enhanced protease activity in the absence of heparin can be constructed by artificially modifying the amino acid sequence in the neighborhood of the reaction site to thereby change the configuration at the reactive site.

If an AT III mutant having an enhanced antithrombin activity in the absence of heparin can be obtained based on the above-mentioned idea, the action of binding to heparin is seemingly not an important characteristic of this AT III mutant. Thus it is conceivable that a reduction in the affinity for heparin caused by introducing an amino acid substitution into the heparin binding site of the above-described AT III mutant would scarcely affect its function, different from the above-mentioned AT III TOYAMA and AT III GENEVA wherein a mutation in the heparin binding site results in abnormalities in the function. It is rather expected that the clinical usefulness of AT III mutant might be enhanced thereby, since interactions with heparin-like substances existing on the surfaces of vascular endothelial cells are suppressed and thus the half-life in the blood is prolonged and the inactivation with neutrophil elastase is avoided.

Based on this idea, the present inventors have conducted extensive studies in order to improve human AT III. As a result, they have successfully constructed the desired novel human AT III mutants, thus completing the present invention.

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Accordingly, the present invention relates to a human antithrombin III (AT III) mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence described in sequence ID No. 2 except that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions.

Namely, the present invention relates to an AT III mutant which is a mutated human AT III characterized in that at least one amino acid in each of four regions of the 11- to 14-positions, the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions has mutated, either singly or combinedly, into another amino acid(s), or an AT III mutant characterized in that in the amino acid sequence of human AT III, one or more amino acid(s) selected from among those at the 11- to 14-positions, the 41- to 49-positions, the 121-to 135-positions and the 384- to 398-positions have mutated into another amino acid(s) and the antithrombin activity in the absence of heparin is elevated as compared with natural AT III.

The human AT III mutant according to the present invention includes the following embodiments:

- (1) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions mutates into another amino acid(s) selected from the group consisting of Ala, Gly, Trp, Pro, Leu, Val, Phe, Tyr, Ile, Glu, Ser, Gln, Asn and Arg.
- (2) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (3) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions and the 41- to 47-positions.
- (4) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions and the 125- to 133-positions.
- (5) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions and the 125- to 133-positions.
- (6) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 11- to 14-positions and that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- (7) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 41- to 47-positions and that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.

- (8) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 125- to 133-positions and that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- (9) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- (10) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) at the 384- to 398-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val, Gly, Arg, Glu and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (11) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) at the 390- to 392-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (12) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (13) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of IIe at the 390- position into Ala, a mutation of Ala at the 391- position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
- (14) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Ala at the 384- position into Gly, a mutation of Ala at the 387- position into Phe, a mutation of Val at the 389-position into Pro, a mutation of Pro at the 397- position into Arg and a mutation of Asn at the 398-position into Glu or Alg is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (15) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into IIe, a mutation of Asp at the 14- position into Ser is present and that an amino acid-(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions.
- (16) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into Ile and a mutation of Asp at the 14- position into Ser, and, another mutation selected from the group consisting of a mutation of Ile at the 390- position into Ala, a mutation of Ala at the 391-position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions and the 125- to 133-positions.
- (17) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 125- position into Gln, a mutation of Arg at the 129- position into Gln, a mutation of Arg at the 132-position into Gln and a mutation of Lys at the 133- position into Asn or Gln is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 384- to 398-positions.
- (18) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 125- position into Gln, a mutation of Arg at the 129- position into Gln, a mutation of Arg at the 133- position into Gln and a mutation of Lys at the 133- position into Asn or Gln, and, another mutation selected from the group consisting of a mutation of Ile at the 390- position into Ala, a mutation of Ala at the 391- position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group

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consisting of the 11- to 14-positions and the 41- to 47-positions.

- (19) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro.
- (20) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- (21) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that IIe-Ala at the 390- to 391-positions mutates into Ala-Leu.
- (22) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Lys at the 125-position mutates into Gln and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- (23) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and IIe-Ala at the 390- to 391-positions mutates into Ala-Leu.
- (24) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- (25) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Lys at the 133-position mutates into Asn and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.

The present invention includes human AT III mutants which are obtained by substituting an amino acid-(s) constituting natural human AT III with another amino acid(s) at a desired position(s).

Each of these human AT III mutants is expressed and produced by using animal cells as a host. As will be described hereinbelow, the mutants thus obtained exhibit elevated antithrombin activities in the absence of heparin as compared with a plasma derived human AT III concentrate or a natural recombinant human AT III. Further, these mutants exert improved drug efficacys in tests with the use of animals as compared with the plasma derived human AT III concentrate. Thus it is expected that they are highly useful for clinical purposes.

The present invention also relates to a DNA coding for the human AT III mutant according to the present invention, an expressible vector which has a DNA containing part or the whole of the DNA sequence coding for the human AT III mutant according to the present invention, a transformant which is obtained by subjecting host cells to transformation with the above-described expressible vector and a method for producing a human AT III mutant which comprises incubating the above-described transformant and recovering the human AT III mutant produced by the transformant from the culture.

The present invention further relates to a drug composition for thrombotic disorders which contains the human AT III mutant according to the present invention and pharmaceutically acceptable carriers, a use of the human AT III mutant according to the present invention for the making of a medicament for treating thrombotic disorders, and a method for treating thrombotic disorders which comprises administering a pharmaceutically effective amount of the human AT III mutant according to the present invention to a patient suffering from the thrombotic disorders.

Further scope and the applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The present invention will be described hereinafter in detail.

The term "AT III" means human AT III in the following description.

Detailed Description of the Invention

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## 1) Isolation of cDNA coding for AT III

Since AT III is mainly synthesized in the liver, a commercially available human liver cDNA library (\lambdagt 11, available from Clonetech) may be used for the isolation of cDNA coding for AT III. Cloning can be effected by a publicly known method. For example, the plaque hybridization method with the use of a synthetic oligonucleotide corresponding to AT III amino acid sequence as a probe [see Sambrook, J. et al., Molecular Cloning, Cold Spring Harbor Laboratory (1989)] may be used therefor.

The clones thus obtained are subcloned into a plasmid such as pUC 18, if required. The nucleotide sequence of cDNA thus obtained can be determined and estimated by the Maxam-Gilbert method [see

Maxam, A.M. and Gilbert, W., Proc. Natl. Acad. Sci. USA. 74, 560 (1977)] or the dideoxy method [Sanger, F., Science, 214, 1205 (1981)]. The nucleotide sequence of the coding region of AT III cDNA thus obtained and the amino acid sequence deduced therefrom are given in SEQ ID. No. 1 in the sequence listing. The amio acid sequence was also described in SEQ ID No. 2 in sequence listing.

### 2) Method for site-directed mutagenesis

Examples of the method for site-directed mutagenesis include a method of Zoller et al. [see Zoller, M. and Smith, M. Methods in Enzymology, 100, 468 (1983)], the one of Kramer et al. [see Kramer, W. and Fritz, H-J, Methods in Enzymology, 154, 350 (1987)] and the one of Vandeyar et al. [see Vandeyar et al., Gene, 65, 129 (1988)].

In the method of Kramer et al., which is called the gapped duplex method, amber mutants of M13 phage such as M13tv18 and M13tv19 are usable as a vector. A DNA coding for AT III is cloned into these vectors. The single-stranded DNA thus obtained and a double-stranded DNA fragment of M13 free from amber mutation (a vector fragment obtained by cleaving M13mpP with Pvu II) are denatured and subjected to degenerative annealing to thereby give a gapped duplex DNA. Next, this DNA is hybridized with a synthetic oligonucleotide having the mutation to be introduced thereinto. After filling up the gap by treating with DNA polymerase and DNA ligase, it was transfected into E. coli mutS strain (BMH71-18 mutS). Then a nonamber phage capable of growing exclusively in supO E. coli is selected. Thus a phage having the desired mutation introduced thereinto can be efficiently obtained. In a practical operation, a commercially available kit (Mutan-G, manufactured by Takara Shuzo Co., Ltd.) may be used. On the other hand, the method of Vandeyar et al. is effected as follows. A single-stranded DNA of M13, into which a DNA.coding for AT III has been cloned, is hybridized with an oligonucleotide having the mutation to be introduced: By using it as a template, dATP, dGTP, dTTP and 5-methyl-dCTP are used as substrates and treated with T7 DNA polymerase. The double-stranded DNA thus formed is treated with T4 DNA ligase to thereby give a closed-circular double-stranded DNA. Next, this double-stranded DNA is treated with a restriction enzyme Msp1 and then with exonuclease III. Thus a circular single-stranded DNA exclusively consisting of a strand having the mutation introduced thereinto is obtained. Then it is transfected into an E. coli (SDM strain) free from any restriction system specific for methylated DNA. Thus the desired clone can be efficiently obtained. In the case of this method, a commercially available kit may be used in practice (T7-GEN In Vitro Mutagenesis Kit, manufactured by United States Biochemical Corporation). The synthetic oligonucleotide having the mutation to be introduced can be synthesized by the phosphoramidite method with the use of a DNA synthesizer (Model 380 A, manufactured by ABI).

### 3) Preparation of template for introducing AT III cDNA mutation

A template for introducing mutation is prepared by inserting restriction sites before and after the coding region of the AT III cDNA obtained in the item 1). The restriction enzymes may be selected from among publicly known ones. In the case of the present invention, a Hind III restriction site was inserted immediately before the coding region of the AT III cDNA while a Bgl II restriction site was inserted immediately thereafter.

First, a plasmid containing the AT III cDNA obtained in the item 1) described above is cleaved with EcoR I and thus a fragment of 1.5 kb including the whole AT III coding region is obtained. This fragment is inserted into a linearized product obtained by cleaving the RF (Replicative Form, a double-stranded DNA) of phage M13tv18 with EcoR I.

Among the clones thus obtained, a single-stranded DNA containing the sense strand of AT III is used as a template. In accordance with the method of Kramer et al., two synthetic oligonucleotides containing the restriction sites of Hind III and BgI II respectively are used as primers and the restriction sites are inserted before and after the coding region of the AT III cDNA.

Subsequently, a fragment containing the AT III cDNA sequence obtained from the clone is inserted into an appropriate plasmid to thereby construct a template for introducing mutation.

In the case of the present invention, a template for introducing mutation can be prepared by inserting the DNA fragment of about 1.5 kb containing the whole AT III coding region, which is obtained by cleaving the above-mentioned clone with Hind III and EcoR I, into the plasmid M13tv19 RF or M13mp19 cleaved with the same enzymes.

Further, the AT III cDNA has a Sac I restriction site (the base part at the 721- to 726-positions in SEQ. ID No. 1) whereby the reactive site can be separated from the heparin binding site. Accordingly, the N-terminal side of AT III obtained by cleaving the above-mentioned clone with Hind III and Sac I, namely, the

DNA fragment containing the heparin binding site is insered into the plasmid M13tv19 or M13mp19 cleaved with the same enzymes. Thus a template for introducing mutation into the heparin binding site can be prepared.

Regarding the reactive site, a similar operation can be carried out by using EcoR I and Sac I.

4) Introduction of mutation into the desired site

In the amino acid sequence of AT III, an amino acid at a desired position can be mutated into another desired amino acid (hereinafter referred to as the desired amino acid) in accordance with the above-mentioned publicly known methods by using a synthetic oligonucleotide containing a DNA coding for the desired amino acid and an appropriate plasmid described in the item 3) as a template. When Gly at the 392-position in AT III is to be mutated into Pro, for example, the AT1R oligonucleotide given in Table 1 may be used. In order to mutate Ala-Gly at the 391-to 392-positions into Phe-Pro, the AT5R oligonucleotide listed in Table 1 may be used. When a number of amino acids separately located are to be mutated, a number of mutations can be introduced by successively effecting the operations for introducing the mutations one by one.

Typical examples of oligonucleotides employed in the present invention are listed in Tables 1 and 2. Amino acid mutation positions and desired amino acids are listed in Tables 3 and 4. Base codons coding for the desired amino acids are not restricted to those listed in Tables 1 and 2 but any codon may be used therefor so long as it codes for the desired amino acid.

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Table 1

Nucleotide sequence of synthetic oligonucleotide for introducing AT III mutation, amino acid to be mutated and position thereof

Oligonucleotide		. Nucleotide sequence	Amino acid to be mutated and its position
AT1R	ັເນ	GTTTAGCGACCGGGGAGCAATCAC 3'	G v392 -pro
AT5R	ນ	GGGGTTTAGCGACCGCGGGAAAATCACAACAGC 3.	Ala391 -Fhe Glv392 -Fro
AT7R	Ŋ	TAGCGAACGGCC <u>GACAGC</u> CACAACAGCGGT 3'	Ile390 -Ala Ala391 -Val
AT9R	5.	CAGCGGTACTGCCAGCTGCTTTC 3'	Ala384 -Gly
AT19R	ហ	ACGGCCAGCAATLGGAACAGCGGTACT 3'	Val389 -Pro
AT24R	5	AATCACAACAAAGGTACTTGCAG 3'	Ala387 -Phe
AT26R	5	GTTTAGCGAACGCGGAATAATCACAACAGC 3'	Ala391 -Ile Gly392 -Pro
AT27R	5	GTTTAGCGAACG <u>CGGACC</u> AATCACAACAG 3'	Ala391 -Gly Gly392 -Pro
AT28R	5	GITTAGCGAACGCGGATAAATCACAACAGC 3'	Ala391 -Tyr Gly392 -Pro
AT29R	S	GTTTAGGGAAGG <u>GGGGAAA</u> TCACAACAGC 3'	Ala391 -Trp Gly392 -Pro
AT30R	ស	GTTTAGCGAACG <u>CGGAAC</u> AATCACAACAG 3'	Ala391 -Val Gly392 -Pro
AT34R	ີດ	TAGCGAACGGCCAATAGCCACAACAGCGGT 3'	Ile390 -Ala Ala391 -Ile
AT35R	ເລ	TAGCGAACGGCCAAGAGCCACAACAGCGCT 3'	Ile390 -Ala Ala391 -Leu
AT38R	່ເວ	TAGCGAACGGCC <u>AAGACC</u> CACAACAGGGG 3'	Ile390 -Gly Ala391 -Leu
AT39R	5	GTTTAGCGAACG <u>GGGAACAGC</u> CACAACAGCGGTA 3'	Ile390 -Ala Ala391 -Val Gly392 -Fro

The underlined part in the nucleotide sequence represents the sequence corresponding to the amino acid to be mutated.

E DESCRIPCIÓ

synthetic oligonucleotide for introducing AT llI mutation, amino acid Table 2 to be mutated and position thereof Nucleotide sequence of 

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Ile390 -Leu Ala391 -Phe Gly392 -Pro Ile390 -Ala Ala391 -Tyr Gly392 -Ala Ala391 -Trp Gly392 -Trp Gly392 mutated Gly392 -- Pro -Arg Asn398 -Arg -Asn Ile390 -Leu Ala391 -Gln Lys133 -Ile Asp 14 Amino acid to be and its position -Leu -61u -G1.n -Leu **-**61n -Arg -61n -Asn 11e390 Arg132 Ala391 Asn398 Pro397 Lys125 Arg129 Arg132 Asn398 Pro 41 Lys 11 Lys133 Lys133 GGGATTCATGGGAATGGATCGTGGGATTGCTGTGCAGAT GTTTAGCGAACGCGGATAAGCCACAACAGCGGTA 3' က . ဗ <del>က</del> ັຕ GTTTAGCGAACGGGGAAAAGCACAACAGCGGTA GTTTAGCGAACGCGGCCAAACAGCGGT GTTTAGCGAACG<u>CGGCCAAAG</u>CACAACCGAGGT GTTTAGCGAACGCGGAAGAATCACAACAGC 3' TTGAAAGTCACCCTCCCGGTTTAGCGAACG TTGAAAGTCACCCGTCGACGGTTTAGCGAACG GGATTTGTTGGCGTTTTGATAGAGTCGGCA 3 GAAAGTCACCTCTCGGGGTTTAGCGAAC 3' CGGCAGTTCAGTTGGGCAAAGAAGA3' Nucleotide sequence GGTGGCCTCCAGGATCTTCTG 3. GTTGGCTTTTTGATAGAGTCG 3' GATAGAGIIGGCAGIICAG 3' TTTGTTGGCGTTTCGATAGAG TTTGTTGGCTTGTCGATAGAG 5 <u>۔</u> 5 ري د 5 5 . . . Oligonucleotide AT40R AT48R AT49R AT46R ATSOR AT2R' AT5R' AT6R' AT1G AT2G AT7G AT8G AT9G ATIF AT2F AT3F

The underlined part in the nucleotide sequence represents the sequence corresponding to the amino acid to be mutated.

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			398 o Asu	1 50						o Asıı		usy o	o Asn	o Asn	пеу а	usy c	usy o	usy o	nsv o	usv o	usv o	nsy c	u V V			
5			Asn Pro	0.10					Asn Pro	Asn Pro	Asn Pro	Asıı Pro	Asn Pro	Asn Pro	Asn Pro	Asn Pro	Asn Pro	Asıı Pro	Asıı Pro	Asn Pro	Asn Pro	Asn Pro	Asu Pro			
			site 395 er Leu	or Leu	ŗ			Ser Leu	er Leu	Ser Leu	Ser Leu	Ser Leu	er Leu	er Leu	Ser Leu	Ser Leu	er Leu	er Leu	er Leu	er Leu	Ser Leu	Ser Leu	Ser Leu	Ser Leu		Ser Leu
10			active I Arg S	Are	714	Ar. 69	Arg	Arg	Arg	Arg	Arg	Arg	Arg S	Arg S	Arg	Arg	Arg S	Arg S	Arg S	Arg S	Arg	Ark	Λrg	Arg	Arg	Λrg
			Re Ala Gly	Alu Pro	Phe Pro		Gly Pro	Tyr Pro	Trp. Pro	Val Pro	ord mar	Val Pro	Phe Pro	Eyr Pro	Tra Pro	Trp Pro	Val Gly	11e 61y	ענט שיו	Leu Gly	лля С1у	Ala Gly	Alu Cly	Ala Gly	Ala Gly	Alu Gly
15			390 Val 11e	Val 11c	Val Ile	e)	Val 11e	Val 11e	Val Ile	Val 11e	Val 11e	Val Ala	Val Leu	Val Ala	Val Ala	Val Leu	Val Ala	Val Ald.	Val Ala	Val Gly	Val 11e ,	Pro 11c	Val Jle	Val. L.Le	Val 11c ,	Vnl Ile
		mutant	Va l	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Va l	Val	Val	Va 1	Val	Va.l.	Val	Val	۷a ]	Vul	Val	Val	Vul
20		III mu	The Ala	Thr Ala	Thr Ala	The Ala	Thr Ala	Thr Ala	Thr Ala	Thr Ala	The Ala	Thr Ala	Thr Ala	Thr Ala	Thr Ala	Thr Ala	Thr Ala		Thr Ala	Thr Ala	Thr Ala	Thr Ala	गाम गाम	The Ala	Thr Ala	Thr Ala
· 25	l.e 3	l in AT	384 Ala Ser	Ala Ser	Alu Ser	Ala Ser	Ala Ser	Ala Ser	Ala Ser	Ala Ser	Ala Ser	Ala Ser	Ala Ser	Ala Ser			Alu Ser				GL <sub>X</sub> Ser	Ala Ser	Ala Ser	Ala Ser	Ala Ser	Alu Ser
	Table	amino acid	133 Lys /	Lys /	Lys ,	Lys /	1	3	s :-:	S :-		3 - 1	s	Lys /	-1 1 1	Lys /			ł	Lys /	Lys {	l.ys 1	1 1 5	Lys 1	Lys /	Lys /
30		Mutated am	132 13 - Arg L	· Arg L	- Arg L	- Arg L	- Arg Ly									- Arg L	- Arg Ly	- Arg L	- Arg L	- Arg Ly						
		Muta	129 Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	ļ	Arg	Arg	Arg	Arg	Vrg	;	Arg	Vrg	Vrg	Arg
35			125 Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Ly's	Lys	Lys	r.	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
40			1.4 Asp 1	l qsA	0	1 dsv		) dsv	1	] dsγ	-	<u>!</u>	;		) )	-	-	;	!	1	:	1 1	1	/sb 1	1	:
40			;	1	SV	:	lsv	:	-	!	1	-	:	1	l l l	-	1 1 1	! !	;	i i	! !	!	-	!	1	dsv
<i>4</i> 5 .			no. 11 III Lys	L,y's	Lys	Lys	Lys	Ly's	Lys	Lys	Ľys	Lys	Lys	Lys	. C.	دې.	Lys:	L.y.s.	S ( ) .	Ly's	Lys	Lys	Lys	Lys	Lys	Lys
			Amino acid r Natural AT 1	118	513	26R	27R	28R	2914	308	46R	X 6 5	4 OK	48K	7 6 6 E	30K	¥ .	7 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	£ 5 5	÷ 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	10 6	76 T	X 4 12	. 214 .	. 2 <u>%</u>	<u>×</u>
50			Natur																							

5		395 Jed Asn Pro Asn	r Leu Asn Pro Asn
10		octive sit I Arg Ser	A Arg Ser Arg Ser Arg Ser B Arg Ser
15		Rer 390 Val ile Ala Gly	11 11e Alu Era 11 11e Phe Era 11 11e Yal Era 11 11e Yal Era 11 11e Alu Era 11 11e Phe Era 11 11e Yal Era 11 11e Phe Era 11 11e Yal Era 11 11e Phe Era 11 11e Yal Era 11 11e Phe Era 11 11e Phe Era 11 11e Yal Era 11 11e Phe Era 11 11e Phe Era 11 11e Yal Era 11 11e Yal Era Ala Leu Gly A
20	III mutant	Thr Ala Val V	
25	Table 4 acid in AT I	J84 - Ala Ser TI	Alu Ser TAlu
30	T Mutated amino a	132 133 Arg Lys	Arg Lys Arg Lys Arg Lys Gln Asn Gln Asn Gln Asn Gln Asn Arg Lys Glu Asn
35	Muta	129 Arg	Arg
40		14 125 - Asp Lys	- ASP GID - ASP GID - ASP GID - ASP GID - ASP Lys - ASP GID
45		- 1	Lys
50		Amino acid no. Natural AT III	161R 165R 1630R 1635R 261R 263R 263R 2F5R 3F5R 7630R 7630R 9635R 9635R 12630R 12630R 12630R

55 5) Combination of mutation in the neighborhood of reactive site and mutation at heparin binding site

As described above, AT III cDNA involves a Sac firestriction site which is located between the reactive site and the heparin binding site. Thus a fragment containing the heparin binding site and another one

containing the reactive site can be obtained by cleaving a plasmid containing the mutated AT III DNA obtained by the method described in the aforementioned item 4) with Hind III and SacI or Sac I and EgI II. An AT III mutant DNA, in which both of the reactive and heparin binding sites have mutated, can be prepared by treating an AT III DNA having a mutated reactive site and another AT III DNA having a mutated heparin binding site, respectively, with restriction enzymes to thereby give a DNA fragment having a mutated reactive site and another DNA fragment having a mutated heparin binding site and connecting the mutated DNA fragments with an appropriate plasmid. According to this method, any combination of mutations at these sites can be achieved. Any plasmid can be used as the one to which the mutated DNA fragments are connected so long as it is suitable for the expression thereof in a host. For example, pSV2 and pK4K are usable.

In Table 4, a symbol 2G35R means a mutant obtained by combining a 2G-mutated DNA fragment with a 35R-mutated one.

## 6) AT III mutant recombinant expression vector and transformant thereof

The DNA coding for the AT III mutant obtained by the above-mentioned method is inserted into an appropriate vector and then the vector obtained is transfected into appropriate host cells. Thus a transformant can be obtained. This transformant is incubated by a conventional method and thus an AT III mutant can be produced in a large amount from the culture.

A DNA coding for an AT III mutant is reconnected to a vector suitable for the expression of the AT III mutant at the downstream of the promoter of the vector by a publicly known method with the use of a restriction enzyme and DNA ligase. Thus a recombinant expression vector can be constructed. The vector is not particularly restricted, so long as it can be replicated and amplified in a host. Neither the promoter nor the terminator is particularly restricted too, so long as they correspond to the host to be used in the expression of the nucleotide sequence coding for the AT III mutant. Thus an appropriate combination thereof may be selected depending on the employed host.

The recombinant expression vector thus obtained is transfected into a host by the competent cell method [see Hanahan, D., J. Mol. Biol., 166, 557 (1983)], the calcium phosphate method [see Wigler, M. et. al., Cell, 11, 222(1977)] and so on to thereby form a transformant. As the host, E. coli, animal cells, etc. are usable. The transformant thus obtained is incubated in a medium suitable for the host. The incubation may be usually carried out at a temperature of from 20 to 45 °C at a pH value of from 5 to 8 with aeration and stirring, if necessary. The AT III mutant can be separated and purified from the culture by combining publicly known separation and purification methods. Examples of these publicly known methods include salting out, solvent precipitation, dialysis, gel filtration, electrophoresis, ion exchange chromatography, affinity chromatography and reversed phase high performance liquid chromatography. The AT III mutant thus obtained has an elevated antithrombin activity in the absence of heparin and an elevated in vivo antithrombotic action in rat as each compared with natural AT III.

### Effects of the Invention

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## (1) Antithrombin activity

By using a Testzym AT III 2 Kit (manufactured by Daiichi Kagaku Yakuhin), the antithrombin activity of the AT III mutant according to the present invention was measured. Namely, the inhibition activity on thrombin thereof in the absence of heparin was measured by using a synthetic substrate (S-2238) of thrombin. As a control, a plasma derived AT III concentrate (Anthrobin P; manufactured by Hoechst Japan) was employed.

In this measurement, a 50 mM Tris hydrochloride buffer solution (pH 7.5) containing 0.1% of bovine serum albumin and 0.15 M of sodium chloride was used. Specimens of various concentrations were reacted with a given amount of thrombin (originated in bovine) at  $37\,^{\circ}$ C for 5 minutes. After the completion of the reaction, the synthetic substrate S-2238 was added and the amount of p-nitroaniline liberated for 2 minutes was determined based on a change in the absorbance at a wavelength of 405 nm. Thus, the remaining thrombin activity was measured. Under these conditions, the AT III mutant concentration at which 50% of the thrombin activity was inhibited (hereinafter referred to as the IC<sub>50</sub>) was calculated.

Table 5 shows the  $IC_{50}$  values of mutants. The  $IC_{50}$  of the plasma derived AT III concentrate in the absence of heparin was  $13.0 \times 10^{-8}$  M and that of the natural recombinant AT III was on almost the same level. In contrast, the  $IC_{50}$  values of the AT III mutants of the present invention were clearly lower than them, suggesting that the antithrombin activity in the absence of heparin had been elevated.

Table 5

		Antithrombin activ	ity of AT III mu	tant
5	Specimen	Antithrombin activity IC <sub>50</sub> × 10 <sup>-8</sup> (M)	Specimen	Antithrombin activity IC <sub>50 × 10<sup>-ε</sup> (M)</sub>
	AT III concentrate	13.0		
	Natural recombinant AT III	14.0		
10	1R	3.0		
10	5R	1.7	38R	6.1
	26R	3.1	9R	5.8
	27R	8.2	19R	8.7
	28R	2.8	24R	10.0
15	29R	1.8	2R'	3.8
,,	30R	2.3	5R'	4.7
	46R	5.0	1G1R	3.7
	39R	5.6	1G5R	2.9
	40R	3.1	2G1R	3.8
20	48R	5.7	2G5R	2.9
20	49R	5.6	2G30R	1.6
	50R	3.0	2G35R	2.2
	7R	2.9	7G5R	1.8
	34R	3.5	9G5R	1.7
25	35R	3.5	127G5R	1.5

## (2) Affinity for heparin

The affinities for heparin of the AT III mutants according to the present invention were compared and examined by the high performance liquid chromatography method with the use of Heparin-5PW (7.5 mm × 75 mm; manufactured by Tosoh Corp.). Namely, a 50 mM Tris hydrochloride buffer solution (pH 7.5) was used as a mobile phase and the concentration of sodium chloride was linearly increased from 0 M to 2 M within 30 minutes at a flow rate of 1 ml/min. The detection was effected based on the absorption at a wavelength of 280 nm and the time required for the elution of each specimen was compared.

As Table 6 shows, the main peak fractions of the AT III and the natural recombinant AT III were eluted, respectively, 22.3 minutes and 23.1 minutes after the intiation of the elution, showing no large difference. Compared with the AT III and the natural recombinant AT III, the mutants having a mutation in the neighborhood of the reactive site showed no remarkable difference. On the other hand, the mutants having mutations in the neighborhood of the reactive site and at the heparin binding site showed each a significantly shortened elution time of the main peak fraction. It was thus confirmed that the introduction of a mutation into the heparin binding site would have lowered the affinity for heparin.

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Table 6

Specimen	Elution time (min)	Specimen	Elution time (min)
AT III concentrate	22.3		
Natural recombinant AT III	23.1		
5R	21.4	9R	22.5
26R	21.2	19R	23.2
28R	22.5	24R	23.4
29R	21.0	5R'	23.6
30R	20.4	1G1R	14.0
46R	17.4	1G5R	14.3
40R	21.0	2G1R	12.5
48R	21.6	2G5R	12.9
7R	21.9	2G30R	13.0
35R	22.1	2G35R	13.1
38R	21.9	7G5R	12.9
		127G5R	10.2

### (3) Antithrombotic action of AT III mutant

By using a plasma derived AT III concentrate (Anthrobin P; manufactured by Hoechst Japan)...and a natural recombinant AT III as controls, the antithrombotic actions of the AT III mutants according to the present invention were measured by the following method.

A method reported by Peters et al. [see Peters, R.F. et al., Thrombosis Haemostasis, 65, 268 (1991)] was modified and employed. Namely, a shunt was formed by cannulating Atom Venous Catheter (4Fr, 3.5 cm, manufactured by Atom) filled with a physiological saline into the carotid arteriovein of a male Sprague-Dawley rat (200 - 300 g) under anesthesia. After blocking the blood stream, the artery side of the shunt was provided with a pulse wave pickup (MPP-3, manufactured by Nippon Koden) and thus changes in the blood stream were monitored with a polygraph recorder during the test period. A calculated amount of a specimen material was diluted with a physiological saline to give a volume of 1 ml and quickly administered once to the rat via the femoral vein. Then the shunt was opened and the blood was allowed to pass. The time required from the point of opening the shunt to the point of the occlusion of the shunt due to the formation of a thrombus was measured and defined as the occlusion time.

Tables 7 and 8 show the results. It was thus proved that the AT III mutants of the present invention had strong antithrombotic actions as compared with the plasma derived AT III concentrate and the natural recombinant AT III.

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Table 7

		Antithrombotic ac	tion of AT III mutant	
5	Specimen	Dose (mg/kg)	Occlusion time Mean ± SD (min)	Case no.
	Physiological saline		21.4± 2.7	11
	AT III concentrate	8 16	29.1± 8.0 36.4±11.6	9
10		32	46,6±14.3	8 8
	Natural recombinant AT III	16 32	39.3±10.5 49.0±13.7	6 4
15	5R	8 16	46.0±18.6 65.7±21.0	7 6
	30R	4 8 16	35.3± 7.1 43.6± 4.7 52.2± 5.3	6 6 6
20	35R	2 4 8	34.7± 5.8 39.9± 9.4 61.4±12.6	7 7 7
25	1G5R	4 8 16	34.1± 8.7 45.2±10.1 69.0±25.7	8 6 6
	2G5R	4 8 16	45.7± 7.5 53.6± 9.3 70.6±11.5	7 9 8
30 .:(**** 	2G30R	4 8 16	35.5± 5.5 45.7±11.2 53.8±13.7	6 6 6
35	2G35R	2 4 8	43.8± 6.6 45.2± 5.8 62.7±28.2	6 6 6

Table 8

	Antithrom	nbotic action of AT III mutant	
Specimen	Dose (mg/kg)	Occlusion time Mean ± SD (min)	Case no
1F5R	4	38.3= 6.0	6
	8	41.3= 7.1	6
	16	54.7±13.1	6
2F5R	4	39.5= 6.1	6
ļ	8	47.8± 9.5	6
	16	59.8±16.1	6
3F5R	4	38.5± 6.3	6
	8	45.3± 5.2	6
	16	55.7± 4.5	6
7G5R	4	36.5± 5.1	6
	8	39.7± 3.9	6
	16	54.2±18.3	6
9G5R	2	38.3= 2.7	6
i	. 4	38.5± 3.1	6
	. 8	49.2± 2.8	6
12G5R	2	36.5± 6.0	6
	4	43.7± 2.7	6
	8	51.2± 6.3	6
127G5R	2	36.0± 7.4	6
	4	46.8 <del>=</del> 4.4	6
	8	57.0±10.9	6

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These results suggest that the AT III mutants according to the present invention serve as anticoagulants and suppress the formation of thrombi. Thus there are expected to be useful as preventive and therapeutic agents for thrombotic disorders.

## (4) Effect of AT III mutant on experimental model of disseminated intravascular coagulation (DIC)

By using a plasma derived AT III concentrate as a control, the effects of the AT III mutants according to the present invention on an experimental model of disseminated intravascular coagulation (DIC) were examined by the following method. A method reported by Sugishima et al., [see Tadashi Sugishima et al., Rinsho to Kenkyu, 62, 274 (1985)] was modified and employed. Namely, a model was formed by cannulating an Alom  $\overline{ ext{Ve}}$ nous Catheter (3Fr, manufactured by Atom) into the jugular vein of a male Sprague-Dawley rat (200 - 300 g) under anesthesia and continuously administering tissue thromboplastin (Thromborel S, manufactured by Behringwerke, AG) for an hour. A test specimen was rapidly administered once via the femoral artery of the rat immediately before starting the administration of tissue thromboplastin. Thirty minutes after the completion of the administration of tissue thromboplastin, the blood was sampled via the descending aorta of the rat and 1/10 volume of 3.8 % sodium citrate was added thereto. After the sampling, 0.5 ml of the blood was immediately taken in a container for an automatic hemocytometer (manufactured by Toa Iyo Denshi K.K.) and platelets were counted with an H.1 System (manufactured by Technicon). The residual blood was centrifuged (3000 rpm, 10 min) to thereby give the plasma. Then fibrinogen contained in the plasma was determined. The content of fibrinogen in the plasma was measured by the thrombin time method (Fibrinogen B-Test Wako, manufactured by Wako Pure Chemical Industries, Ltd.).

Table 9 shows the results. Thus it was found out that the AT III mutants of the present invention exerted strong effects on a decrease in platelet count and the reduction of plasma fibrinogen level in the experimental DIC model induced with tissue thromboplastin as compared with the plasma derived AT III concentrate. Based on these results, the AT III mutants of the present invention are expected as a useful therapeutic agent for DIC.

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#### Table 9

Effect of AT	III mutant o	on experim	ental DIC model	
Specimen	Dose (mg/kg)	No. of cases	Platelet count (x 10³/µl) Mean ± SD	Amount of plasma fibrinogen (g/l) Mean ± SD
Physiological saline (no tissue thromboplastin administered)		12	952.7±110.6	1.95±0.15
Sole administration of tissue thromboplastin		12	424.1±122.3	0.12±0.03
AT III concentrate	8	12	527.0±108.8	0.17±0.06
	16	11	596.6± 60.9	0.20±0.07
	32	12	683.7±128.9	0.77±0.41
1G5R	4	6	574.7± 54.2	0.36±0.42
	8	6	729.7± 77.6	0.99±0.54
2G5R	4	6	618.5±116.1	0.21±0.07
	8	6	618.2±146.3	0.77±0.28
1F5R	4	6	557.2±154.4	0.30±0.34
	8	6	649.5±112.6	0.64±0.39
2F5R	4	6	528.8± 89.1	0.24±0.08
	8	5	659.8± 53.6	0.63±0.24
3F5R	4	6	487.3± 83.4	0.16±0.08
	8	6	664.5± 61.5	0.54±0.37

This AT III mutant can be orally, topically, intravenously, intramuscularly or subcutaneously administered, among which topical or intravenous administration is preferable. The dose may range from 0.1 to 100 mg/kg and preferably from 0.5 to 20 mg/kg, and is determined depending on the body weight of the patient. It is dissolved in from 1 to 50 ml of a physiological saline and used.

It may be formulated into, for example, wettable powders, solutions, tablets, capsules, powders, suppositories and the like. As carriers for formulating these preparations, pharmaceutically acceptable fillers, disintegrating agents, lubricants and dispersion media commonly employed in the art may be used.

## Brief Description of the Drawings

Fig. 1 is a figure showing a process for constructing pKCRNK.

Fig. 2 is a figure showing a process for constructing pUC19st<sup>-</sup>Ad.

Fig. 3 is a figure showing a process for constructing pAdPst-.

Fig. 4 is a figure showing a process for constructing pKCRNKAd.

Fig. 5 is a figure showing a process for constructing pKCR5H3B.

Fig. 6 is a figure showing a process for constructing pKCR5H3BAd.

Fig. 7 is a figure showing a process for constructing pKCRAdEcoB<sup>-</sup>H<sup>-</sup>.

Fig. 8 is a figure showing a process for constructing pKNK.

Fig. 9 is a figure showing a process for constructing pK4K.

Fig. 10 is a figure showing a process for constructing pKCR5RAd.

### Examples

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To further illustrate the present invention in detail and concretely, the following Examples will be given, though it is to be understood here that the present invention is never restricted thereto.

### Example 1

Cloning of DNA sequence coding for AT III

By the use of a commercially available human liver cDNA library (\(\lambda\gamma\) 11, available from Clonetech) as a starting material, screening was effected by a conventional method with a \$2\text{P-labeled} synthetic oligonucleotide as a probe. The sequence of the synthetic oligonucleotide comprised the nucleotide sequence corresponding to the amino acids at the 314- to 322-positions of AT III based on the report by Chandra et al.

As the result of the screening, two clones #2 and #6 were obtained. DNA fragments were collected from each clone by using a restriction enzyme EcoR I and subcloned into M13mp18 to thereby determine the nucleotide sequence. As a result, it was confirmed that the clone #2 contained a fragment of about 1.3 kb corresponding to the sequence of the 33rd amino acid to polyA, while another clone #6 contained a fragment of about 1.1 kb corresponding to the initiation codon to the 348th amino acid. Subsequently, inserts were excised from these clones by using EcoR I and each of the inserts was subcloned into pUC18 cleaved with EcoR I. Thus pUC-H and pUC-L were prepared respectively from the clones #2 and #6.

Next, a DNA fragment of about 3.7 kb (containing a sequence of about 1.0 kb corresponding to pUC18 and the N-terminal side of antithrombin III), which was obtained by cleaving pUC-L with Nco I and Hind III, was connected to another DNA fragment of about 0.5 kb (containing a sequence corresponding to the C-terminal side of antithrombin III) which was obtained by cleaving pUC-H with Nco I and Hind III. Thus a plasmid AT III FLpUC containing all coding regions ranging from the initiation codon to the terminator codon of AT III was obtained. The whole sequence from the initiation codon to the terminator codon of the AT III cDNA contained in this plasmid was represented by SEQ ID No. 1 in the sequence listing.

#### Example 2

Insertion of restriction site

By the use of the plasmid AT III FLpUC obtained in the above Example 1 as a starting material, a DNA having a Hind III restriction site inserted immediately before the AT III coding sequence and a BgI II restriction site immediately thereafter was prepared. First, AT III FLpUC was cleaved with EcoR I to thereby give a fragment of about 1.5 kb containing the whole AT III coding region. This fragment was inserted into the above-mentioned one obtained by cleaving RF of M13tv18 with EcoR I to linearize. Among the clones thus obtained, a clone giving the sense strand of AT III as a single-stranded DNA was referred to as tvATR. By using the single-stranded DNA of this tvATR as a template and the two synthetic oligonucleotides given below, each containing the restriction site of each enzyme, as a primer, the restriction sites were introduced in accordance with the method of Kramer et al.

5' TACATGGCCGAAGCTTCGTAATCAT 3'.

### to AT3B 29mer:

5' CAAAGAATAAGATCTTATTACTTAACACA 3'.

In the practical operation, a commercially available kit (Mutan G, manufactured by Takara Shuzo Co., Ltd.) was used. Namely, about 0.5 µg of the single-stranded DNA of tvATR and 0.2 µg of dsDNA contained in the kit (obtained by cleaving the RF DNA of a phage M13mpP lacking in a Pvu II fragment containing the multiple-cloning site of M13mp18 with Pvu II to linearize) were allowed to stand in 20 mM Tris. HCl pH 8 -10 mM MgCl<sub>2</sub> - 50 mM NaCl - 1 mM DTT at 100 °C for 3 minutes, at 65 °C for 10 minutes and at 37 °C for 10 minutes to thereby form a gapped duplex. A 1/10 portion of this gapped duplex was collected and mixed with 5 pmol portions of AT5H and AT3B the 5'-end of which had been substituted with phosphate with T4 polynucleotide kinase, and the resulting mixture (3 μl in total) was allowed to stand at 65 °C for 15 minutes and at 37°C for 15 minutes. Next, 25 µl of a buffer solution contained in the kit [50 mM Tris+HCl pH 8 - 60 mM ammonium acetate - 5 mM MgCl<sub>2</sub> - 5 mM DTT - 1 mM NAD - 0.5 mM each of dNTPs (A, C, G, T)], 60 U of E. coli DNA ligase and 1 U of T4 DNA polymerase were added thereto and the resulting mixture was allowed to stand at 25 °C for about 2 hours. After adding 3 µl of 0.2 M EDTA (pH 8) and heating at 65 °C for 5 minutes, part of the mixture was collected and transfected into competent cells of an E. coli BMH71-18mutS strain prepared by the method of Hanahan [see Hanahan, D., J. Mol. Biol., 166, 557 (1983)]. Plaques obtained by using an E. coli MV1184 strain as an indicator were picked and incubated by a conventional method to thereby give an RF DNA. This DNA was cleaved with restriction enzymes Hind III and BgI II and the nucleotide sequence of a clone having a new restriction site was determined by the

dideoxy method. Thus it was confirmed that the desired mutation had been introduced. The clone thus obtained was referred to as AT5H3B.

A DNA fragment of about 1.5 kb obtained by cleaving this AT5H3B with Hind III and EcoR I was inserted into M13lv19RF which had been subjected to linearize by similarly cleaving with Hind III and EcoR I. The clone thus obtained was referred to as tv19-5H3B. A DNA fragment obtained by cleaving a plasmid pSV2-dhfr [see Lee, F. et al., Nature, 294, 228 (1981); Subramani, S. et al., Mol. Cell. Biol., 1, 854 (1981)] with Hind III and Bgl II and eliminating a region coding for mouse dihydrofolate reductase (dhfr) was connected to another DNA fragment obtained by cleaving AT5H3B with Hind III and Bgl II too and containing the whole AT III coding region. Thus a plasmid pSV2-5H3B was obtained. Further, a DNA fragment of about 730 bp obtained by cleaving pSV2-5H3B with Hind III and Sac I and coding for the N-terminal side of AT III was inserted into M13tv19 and M13mp19 which had been subjected to linearize by cleaving with Hind III and Sac I to thereby respectively give tv19-ATN and mp19-ATN.

#### Example 3

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## a) Preparation of 1R mutant DNA

A sequence coding for an AT III mutant 1R wherein the 392nd Gly of AT III had been substituted with Pro (Table 3) was obtained by the site-directed mutagenesis method. Namely, in accordance with the method of Kramer et al., the single-stranded DNA of AT5H3B obtained in Example 2 was used as a template and treated with a synthetic oligonucleotide AT1R (Table 1) to thereby give the desired clone 1Rmut. The operation was effected by using a commercially available kit (Mutan G) by the same method as the one described in Example 2.

Twelve plaques thus obtained were picked up and analyzed. As a result, five of these clones were found to be the desired ones. The RF DNA of the obtained clone was cleaved with Hind III and Bgl II and the DNA fragment of about 1.4 kb thus obtained was replaced with a mouse DHFR gene in a plasmid pSV2-dhfr, similar to the procedure employed in Example 2, to thereby construct a plasmid pSV2-1R.

## b) Preparation of other DNAs having mutation in the neighborhood of the reactive site

In order to introduce a mutation in the neighborhood of the reactive site other then 1R, the abovementioned method of Kramer et al. was effected by using tV19-5H3B obtained in Example 2 as a template. Thus mutations of 5R, 26R, 28R, 29R, 30R, 39R, 40R, 46R, 48R, 49R, 50R, 27R, 7R, 34R, 35R, 38R, 9R, 19R, 24R, 2R', 5R' and 6R' were introduced. The amino acid sequence in the neighborhood of the reactive site of each of these AT III mutants is given in Table 3, while the sequences of synthetic oligonucleotides employed for the introduction of the mutations are listed in Tables 1 and 2. Similar to the procedure described in Example 2, a reaction for introducing a mutation was performed in accordance with the manual accompanying the kit and several clones thus formed were collected. Then the nucleotide sequences were determined and thus the clones having the desired mutation introduced thereinto were obtained. From each clone, a DNA fragment of about 1.4 kb was obtained by using Hind III and Bgl II. In the cases of 5R, 26R, 28R, 30R, 27R, 7R, 19R, 24R, 2R', 5R' and 6R', the obtained fragments were replaced with a mouse DHFR gene in pSV2-dhfr in the same manner as those described in Example 2 and Example 3 a) to thereby respectively give plasmids pSV2-5R, pSV2-26R, pSV2-28R, pSV2-30R, pSV2-27R, pSV2-7R, pSV2-19R, pSV2-24R, pSV2-2R', pSV2-5R' and pSV2-6R'. In the cases of 39R, 40R, 46R, 48R, 49R, 50R, 34R, 35R and 38R, on the other hand, each of the DNA fragments was replaced with a part of an NKAF gene in a plasmid pK4K which will be described hereinbelow to thereby respectively give plasmids pK4K-39R, pK4K-40R, pK4K-46R, pK4K-48R, pK4K-49R, pK4K-50R, pK4K-34R, pK4K-35R and pK4K-38R. In the cases of 29R and 9R, DNA fragments of about 1.4 kb were isolated again from plasmids pSV2-29R and pSV2-9R by using Hind III and BgI II and plasmids pK4K-29R and pK4K-9R were constructed by the same method as those described above.

### Example 4

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## Preparation of heparin binding site-mutated DNA

Among mutations at the heparin binding site, the mutations of 1G, 2G and 8G were introduced in accordance with the method of Kramer et al. by using tv19-5H3B obtained in Example 2 as a template. The sequences of synthetic oligonucleotides employed therein are given in Table 2. The clones having the

desired mutation introduced thereinto were referred to as 1Gmut, 2Gmut and 8Gmut respectively. From these clones, DNA fragments of about 1.4 kb were excised by using Hind III and BgI II and treated by the same method as the one employed in the cases of the mutations at the reactive site. Thus plasmids pSV2-1G, pSV2-2G and pSV2-8G were obtained. Further, a DNA fragment of about 730 bp obtained by cleaving pSV2-1G with Hind III and Sac I was inserted into M13Iv19 cleaved with the same enzymes to thereby give tv19-1GN.

The mutations of 1F, 2F, 3F and 7G were introduced in the same manner by using tv19-ATN obtained in Example 2 as a template. The M13 clones having the desired mutation introduced thereinto were referred to as 1Fmut, 2Fmut, 3Fmut and 7Gmut, respetively.

The mutation of 9G was introduced in accordance with the method of Vandeyar et al. with the use of mp19-ATN as a template. The practical operation was performed in accordance with the manual accompanying a kit (T7-GEN In Vitro Mutagenesis System available from USB). First, 1 µg of mp19-ATN single-stranded DNA and 2 pmol of a synthetic oligonucleotide AT9G, the 5'-end of which had been substituted with phosphate with T4 polynucleotide kinase, were heated at 65 °C in 40 mM Tris-HCl pH 7.5 - 20 mM MgCl<sub>2</sub> - 50 mM NaCl for 5 minutes and then slowly cooled to room temperature. To this reaction mixture (10 µl) were added 2 µl of 10 X Synthesis mix (100 mM Tris-HCl pH 7.5 - 20 mM DTT - 5 mM dATP - 5 mM dGTP - 5 mM dTTP - 5 mM 5-methyl-dCTP - 10 mM ATP), 2.5 U of T7 DNA polymerase and 5 U of T4 DNA ligase to thereby give a final volume of 20 µl, followed by allowing to stand at 37°C for 1 hour. Thus an RF DNA, in which the strand having a mutation introduced thereinto had been exclusively methylated, was synthesized. After inactivating the enzyme by heating the reaction mixture at 70°C for 10 minutes, 5 U portions of restriction enzymes Msp I and Hha I were added and allowed to react at 37°C for 45 minutes. Thus one of the DNA strands of the double-stranded DNA used as a template which had not been methylated was exclusively nicked with Msp I and the template single-stranded DNA which had not been replicated into the double-stranded one was cleaved with Hha I.

Subsequently, 50 U of exonuclease III was added to the reaction mixture and allowed to react at 37 °C for 45 minutes. Then only the nicked template strand was digested and, as a result, the DNA strand having mutation introduced thereinto was concentrated. After ceasing the reaction by heating at 70 °C for 10 minutes, the reaction mixture was transfected into an E. coli SDM strain (mcrAB) free from any restriction system specific for methylated DNA by an ordinary method. Several plaques thus obtained were picked up and DNAs were obtained. Then the nucleotide sequences thereof were determined and thus a clone having the desired mutation introduced thereinto was selected. From the clone thus obtained, a DNA fragment of about 730 bp was isolated by using Hind III and Sac I and inserted into pSV2-5H3B which had been cleaved with the same enzymes to thereby eliminate fragments of the same size. Thus pSV2-9G was obtained.

The 12G mutant was obtained by further introducing a mutation by using a synthetic oligonucleotide AT2G (Table 2) with the use of a DNA having the mutation of 1G introduced thereinto as a template. Namely, it was obtained in accordance with the method of Kramer et al. by using tv19-1GN as a template. After confirming that the desired mutation had been introduced, the obtained clone was referred to as 12Gmut.

The 127G mutant was obtained in accordance with the above-mentioned method of Vandeyar et al. by using a single-stranded DNA of 12Gmut as a template and treating with a synthetic oligonucleotide AT7G. After confirming that the desired mutation had been introduced, the obtained clone was referred to as 127Gmut.

### 45 Example 5

Preparation of DNA having mutations both in the neighborhood of the reactive site and at the heparin binding site

## a) Preparation of 1G5R mutant DNA

A DNA of the 1G5R mutant having a combination of a mutation 1G at the heparin binding site with another mutation 5R in the neighborhood of the reactive site was constructed in the following manner.

The RF DNA of 1Gmut obtained in Example 4 was cleaved with Hind III and Sac I and thus a DNA fragment of about 730 bp having a mutation at the heparin binding site was prepared. The pSV2-5R obtained in Example 3 was cleaved with Sac I and Bgl II and thus a DNA fragment of about 670 bp having a mutation in the neighborhood of the reactive site was prepared. These DNA fragments were combined together and inserted into pSV2-dhfr from which a mouse DHFR gene had been eliminated by using Hind III

and BgI II. Thus pSV2-1G5R was constructed. Further, this pSV2-1G5R was cleaved with Hind III and BgI II and a DNA fragment of about 1.4 kb thus formed was inserted into a plasmid which was obtained by eliminating a part of a NKAF gene in a plasmid pK4K as will be described hereinafter by cleaving the plasmid pK4K with Hind III and BamH I. Thus pK4K-1G5R was constructed. The preparation of these DNA fragments having mutation and the construction of pSV2-1G5R and pK4K-1G5R by combining these mutated DNA fragments were performed in accordance with publicly known methods. E. coli HB101-pK4K-1G5R containing the plasmid pK4K-1G5R has been deposited with Fermentation Research Institute of Agency of Industrial Science and Technology of the Ministry of International Trade and Industry under the accession number of FERM BP-3806, on March 26, 1992.

## b) Preparation of 2G5R mutant DNA

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A DNA of the 2G5R mutant having a combination of a mutation 2G at the heparin binding site with another mutation 5R in the neighborhood of the reactive site was constructed in the following manner.

The RF DNA of 2Gmut obtained in Example 4 was cleaved with Hind III and Sac I and thus a DNA fragment of about 730 bp having a mutation at the heparin binding site was prepared. The pSV2-5R obtained in Example 3 was cleaved with Sac I and BgI II and thus a DNA fragment of about 670 bp having a mutation in the neighborhood of the reactive site was prepared. These DNA fragments were combined together and inserted into pSV2-dhfr from which a mouse DHFR gene had been eliminated by using Hind III and BgI II. Thus pSV2-2G5R was constructed. Further, this pSV2-2G5R was cleaved with Hind III and BgI II and a DNA fragment of about 1.4 kb thus formed was inserted into a plasmid which was obtained by eliminating a part of a NKAF gene in a plasmid pK4K as will be described hereinafter by cleaving the plasmid pK4K with Hind III and BamH I. Thus pK4K-2G5R was constructed. E. coli HB101-pK4K-2G5R containing the plasmid pK4K-2G5R has been deposited with Fermentation Research Institute of Agency of Industrial Science and Technology of the Ministry of International Trade and Industry under accession number of FERM BP-3807, on March 26, 1992.

## c) Preparation of other both site-mutated DNAs

The DNAs each having a mutation at the corresponding site obtained in Examples 3 and 4 were employed. As a DNA fragment having a mutation at the heparin binding site, DNA fragments of about 730 bp obtained by cleaving pSV2-1G, pSV2-2G, pSV2-9G, 1Fmut, 2Fmut, 3Fmut, 7Gmut, 12Gmut and 127 Gmut each with Hind III and Sac I were prepared. Separately, as a DNA fragment having a mutation at the reactive site, DNA fragments of about 670 bp were obtained by cleaving pSV2-1R, pSV2-5R (or pSV2-1G5R) and pSV2-30R each with Sac I and BgI II. Further, pK4K-35R was cleaved with Sac I and Xho II to thereby give a DNA fragment of about 670 bp (In a DNA prepared by inserting an AT III mutant gene with a Hind III-Bgl II fragment into a plasmid wherein a part of an NKAF gene had been eliminated from pK4K by cleaving with Hind III and BamH I, the BgI II-cleaved end is connected to the BamH I-cleaved end. Thus it is impossible to cleave this DNA again with Bgl II. However, this site can be cleaved with Xho II.). These DNA fragments were combined together and then inserted into a plasmid wherein a part of the NKAF gene had been eliminated from pK4K by cleaving with Hind III and BamH I. Thus pK4K-1G30R, pK4K-1G35R, pK4K-2G30R, pK4K-2G35R, pK4K-1F5R, pK4K-2F5R, pK4K-3F5R, pK4K-7G30R, pK4K-7G35R, pK4K-9G5R, pK4K-9G30R, pK4K-9G35R, pK4K-12G5R, pK4K-12G30R, pK4K-12G35R, pK4K-12TG5R, pK4K-127G30R and pK4K-127G35R were constructed. Furthermore, pSV2-1G1R and pSV2-2G1R were constructed in a similar manner by using pSV2-dhfr from which a mouse DHFR gene had been eliminated with the use of Hind III and BgI II.

## Example 6

50 Construction of expression vector for animal cells

## a) Construction of natural recombinant AT III and 1R expression vector

A plasmid pNK8308 (disclosed in European Patent Publication-A3 No. 357067) containing a cDNA coding for recombinant natural killer cell activating factor (NKAF) was digested with Bgl II and BamH I and electrophoresed on an agarose gel. Thus an NKAF cDNA fragment of about 0.75 kb was isolated. A plasmid pKCR [see O Hare, K. et al., Proc. Natl. Acad. Sci. USA, 78, 1527 (1981)] was digested with BamH I and dephosphorylated with alkaline phosphatase. The vector DNA thus obtained was connected (ligated) to the

NKAF cDNA fragment by adding T4 DNA ligase to thereby give pKCRNK (Fig. 1).

A plasmid pUC19 was digested with Pst I, then treated with T4 DNA polymerase by a conventional method to thereby blunt (to thereby be blunt-ended) both of the 3'- and 5'-ends and then ligated, thus giving pUC19Pst\*. Subsequently, this pUC19Pst\* was digested with BamH I and dephosphorylated with alkaline phosphatase. The vector DNA thus obtained was ligated with a DNA fragment of about 2.4 kb [containing adenovirus promoter, mouse dihydrofolate reductase (DHFR) gene and SV40 polyA signal], which had been isolated by digesting a plasmid pAdD26SV(A) (no.3) [see Kaufmann, R. and Sharp, P., Mol. Cell. Biol., 2, 1304 (1982)] with BamH I and electrophoresing on an agarose gel, to thereby give pUC19Pst-Ad (Fig. 2). Further, this pUC19Pst<sup>-</sup> Ad was digested with Pst I and blunt-ended with T4 DNA polymerase and then ligated to thereby give pUC19Pst<sup>-</sup> AsPst<sup>-</sup>. Then a DNA fragment of about 2.9 kb containing a tetracycline-resistant gene, which had been isolated by digesting pAdD26SV(A) (no. 3) with BamH I, dephosphorylating and electrophoresing on an agarose gel, was ligated with another DNA fragment of about 2.4 kb containing adenovirus promoter, mouse DHFR gene and SV40 polyA signal, which had been isolated by digesting pUC19Pst- AdPst- with BamH I and electrophoresing on an agarose gel, to thereby give pAdPst<sup>-</sup> (Fig. 3). Then the pAdPst<sup>-</sup> was digested with EcoR I and blunt-ended by treating with a DNA polymerase I Klenow fragment. Subsequently, it was digested with Pst I and blunt-ended with T4 DNA polymerase. Then Aat II linker was added thereto and ligated therewith and the obtained product was digested with Aat II and electrophoresed on an agarose gel. Thus a DNA fragment of about 2.7 kb containing adenovirus promoter, mouse DHFR gene and SV40 polyA signal was obtained. This DNA fragment was ligated with a DNA obtained by digesting pKCRNK with Aat II and dephosphorylating to thereby give pKCRNKAd (Fig. 4).

The plasmid pSV2-5H3B obtained in Example 2 was digested with Hind III and Bgl II and a DNA fragment of about 1.4 kb containing AT III cDNA was isolated. This fragment was ligated with a vector DNA obtained by digesting a plasmid pIC19R [see Marsh, J.L. et. al., Gene, 32, 481 (1984)] with Hind III and Bgl II to thereby give pIC19R5H3B. Next, this pIC19R5H3B was digested with BamH I and Bgl II and a DNA fragment of about 1.4 kb containing 5H3B cDNA was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCR with BamH I and dephosphorylating to thereby give pKCR5H3B (Fig. 5).

pKCRNKAd was digested with Aat II and a DNA fragment of about 2.7 kb containing adenovirus promoter, mouse DHFR gene and SV40 polyA signal was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCR5H3B with Aat II and dephosphorylating to thereby give pKCR5H3BAd (Fig. 6). The pKCR5H3BAd was used in order to express a natural recombinant AT III in animal cells as will be described in Example 7.

Similarly, by the use of pSV2-1R obtained in Example 3 a) as the starting material, pKCR1RAd was obtained. The pKCR1RAd was used in order to express a mutant 1R in animal cells as will be described in Example 7.

### b) Construction of expression vectors of various mutants in animal cells

pKCR5H3BAd was digested with EcoR I and then self-ligated. Thus pKCRAdEco wherein SV40 promoter, a part of the NKAF gene and a part of rabbit β-globin gene had been eliminated was selected. The pKCRAdEco was digested with BamH I, blunt-ended with a DNA polymerase I Klenow fragment and then ligated to thereby give pKCRAdEcoB<sup>-</sup>. Subsequently, the pKCRAdEcoB<sup>-</sup> was digested with Hind III, blunt-ended with a DNA polymerase I Klenow fragment and then ligated to thereby give pKCRAdEcoB<sup>-</sup>H<sup>-</sup> - (Fig. 7).

pKCRNKAd was digested with Hind III and BamH I and a DNA fragment of about 0.4 kb containing a part of the NKAF gene was isolated. Then it was ligated with a vector DNA obtained by digesting pIC19R with Hind III and BamH I to thereby give pIC19RNKK. The pIC19RNKK was digested with BgI II and BamH I and a DNA fragment of 0.4 kb containing a part of the NKAF gene was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCR with BamH I and dephosphorylating to thereby give pKNK (Fig. 8).

pKNK was partially digested with EcoR I and a DNA fragment of about 1.5 kb containing SV40 promoter, a part of NKAF gene and a part of rabbit \$-globin gene was isolated. Then this DNA fragment was ligated with a vector DNA obtained by digesting pKCRAdEcoB<sup>+</sup>H<sup>-</sup> with EcoR I and dephosphorylating to thereby give pK4K (Fig. 9).

As Fig. 9 shows, pK4K contains the promoter of an early gene of SV40, the replication initiation region of SV40, a part of the NKAF gene, a part of the rabbit  $\beta$ -globin gene (splicing and polyA signal), the polyA signal of the early gene of SV40, the major late gene promoter and the 5' splice signal of type II adenovirus, rabbit immunoglobulin 3' splice signal, mouse DHFR gene, the polyA signal of the early gene of SV40, the

replication initiation region of pBR322 and a  $\beta$ -lactamase gene originating in pBR322 (Amp  $\gamma$ ) and the dhir was connected on the downstream side of the major late gene promoter of adenovirus and a part of the NKAF gene was connected on the downstream side of the promoter of the early gene of SV40.

An expression vector in animal cells can be constructed by inserting an AT III mutant gene into a site remaining after excising a part of the NKAF gene of pK4K with Hind III and BamH I. In practice, expression vectors of the mutants 1G5R and 2G5R were prepared by using pSV2-1G5R and pSV2-2G5R respectively and pK4K by the above-mentioned method as shown in Example 5 a) and b). These vectors were referred to as pK4K-1G5R and pK4K-2G5R. As described in Example 3 b) and Example 5 c), expression vectors of other mutants were similarly constructed by using pK4K.

c) Construction of expression vectors of mutants 5R and 7R

The plasmid pSV2-5R obtained in Example 3 b) was digested with Hind III and BgI II and thus a DNA fragment of about 1.4 kb containing a 5R gene was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKNK with Hind III and BamH I to thereby eliminate a part of the NKAF gene, thus giving pKNK5R. The pKNK5R was digested with EcoR I and a DNA fragment of about 1.5 kb containing the promoter of the early gene of SV40, a 5R gene and a part of the rabbit β-globin gene was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCRAdEcoB<sup>+</sup>H<sup>-</sup> with EcoR I and dephosphorylating to thereby give pKCR5RAd (Fig. 10). Similarly, pKCR7RAd was obtained by using pSV2-7R obtained in Example 3 b).

Example 7

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Expression of AT III mutant by animal cells

a) Expression by CHO cell

CHO cells [dhfr-deficient strain, see Urlaub, G. and Chasin, L.A., Proc. Natl. Acad. Sci. USA, 77, 4216 (1980)] were inoculated in an incubation flask at a ratio of  $7 \times 10^5$  cells/5 ml/the flask of 25 cm $^2$ . On the next day, 3 µg of the plasmid pKCR1RAd obtained in Example 6 a) was transfected by the calcium phosphate method with the use of a CellPhect (a kit manufactured by Pharmacia). As a medium, one obtained by adding fetal calf serum to a 1:1 mixture (a DF medium) of Ham F12 medium with Dulbecco's modified Eagle medium in such a manner that the obtained medium contained 10% of the fetal calf serum. was used. After 3 days, the cells were trypsinized and diluted with a selection medium (DF medium free from hypoxanthine and thymidine + 10% dialyzed fetal calf serum). Then 1 ml portions of the cells contained in one incubation flask (25 cm²) were pipetted into each of wells of four 24-well plates for incubation and the incubation was continued in the selection medium while replacing the medium with a fresh one at intervals of 3 to 4 days. Cells surviving under these conditions were those transformed by the mouse DHFR gene. After approximately 2 weeks, the colonies thus formed were dispersed by trypsinizing in wells and a fresh medium was added, followed by incubating for additional 3 to 4 days. Then the culture broth was exchanged and the amount of 1R contained in the culture supernatant was determined by the EIA method on the next day. Each clone showing an expression yield of about several ten ng/ml/day or more was transinoculated into a selection medium containing 50 nM of methotrexate (MTX) and incubated for 2 to 3 weeks. Further, the MTX concentration was successively elevated to 100 nM, 400 nM and 1000 nM and 45 the incubation was continued in the same manner. Among clones growing at the MTX concentration of 1000 nM, those showing high expression yields were cloned by the limiting dilution method with the use of a 96well plate. In the state of confluent growth, a clone 110-6, which was a typical example of those thus obtained, secreted about 10 µg/ml/day of 1R into the culture supernatant at 0.3 ml of the medium/cm². Similarly, CHO cells capable of expressing a natural recombinant AT III were obtained by using pKCR5H3BAd obtained in Example 6 a).

- b) Expression of various mutants by BHK cell
- i) Use of pSV2 vectors

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The plasmids shown in Fxamples 3 and 5, which were constructed by replacing the mouse DHFR gene in a plasmid pSV2-dhfr by an AT III mutant DNA, can be used for expressing various mutants by transfecting into animal cells together with pSV2-dhfr (cotransfection).

BHK cells [tk-ts13 strain, see Waechter, D.E. and Baserga, R., Proc. Natl. Acad. Sci. USA, 79, 1106 (1982)] were inoculated in an incubation flask at a ratio of 5 × 10<sup>5</sup> cells/5 ml/the flask of 25 cm<sup>2</sup>. On the next day, 7 μg of a plasmid pSV2-28R having a gene of a mutant 28R shown in Example 3 b) introduced thereinto was transfected into the BHK cells together with 3.5 μg of pSV2-dhli by the calcium phosphate method with the use of CellPhect. As a medium, one obtained by adding fetal calf serum to Dulbecco's modified Eagle medium in such a manner that the obtained medium contained 5% of the fetal calf serum, was used. After 3 days, the cells were trypsinized and subcultured into a 75 cm² incubation flask with a medium containing 200 nM of MTX. After incubating for 10 days while replacing the medium with a fresh one at intervals of 2 to 3 days, the cells were subcultured into a 175 cm² incubation flask with a medium containing 1000 nM of MTX. After incubating for additional 10 days while replacing the medium with a fresh one at intervals of 2 to 3 days, a cell strain showing a high expression yield was cloned by the limiting dilution method with the use of a 96-well plate. A clone #4 thus obtained secreted about 0.7 μg/ml/day of 28R into the culture supernatant at 0.3 ml of the medium/cm² in a state of confluent growth. Regarding the plasmids containing other mutant DNAs which were constructed with pSV2 and described in Examples 3 and 5, expression cells could be obtained by the same method as the one described above.

#### ii) Use of other vectors

BHK cells (tk<sup>-</sup>ts13 strain) were inoculated in an incubation flask at a ratio of 3 × 10<sup>5</sup> cells/5 ml/the flask of 25 cm². On the next day, 3 μg of a plasmid pK4K-2G5R having a gene of a mutant 2G5R obtained in Example 5 b) introduced thereinto was transfected into the BHK cells by the calcium phosphate method with the use of CellPhect. As a medium, one obtained by adding fetal calf serum to Dulbecco's modified Eagle medium in such a manner that the obtained medium contained 5% of the fetal calf serum, was used. After 2 days, the cells were trypsinized and diluted with a medium containing 250 nM of MTX. The cells in one 25 cm² incubation flask were pipetted into wells of twelve 24-well plates for incubation. Then the incubation was continued while replacing the medium with a fresh one at intervals of 3 to 4 days. After 12 days, the colonies thus formed were dispersed by trypsinizing in the wells and the medium was added. After incubating for additional 6 days, the culture broth was exchanged. On the next day, the amount of each mutant contained in the culture supernatant was measured by the EIA method and a cell strain showing a high expression yield was cloned. A clone 6-5 thus obtained secreted about 16 μg/ml/day of 2G5R into the culture supernatant at 0.3 ml of the medium/cm² in a state of confluent growth.

Regarding pKCR1RAd, pKCR5RAd and pKCR7RAd described in Example 6 and the plasmids containing other mutant DNAs described in Examples 3 and 5, which were constructed by using pK4K, expression cells were obtained in a similar manner. Further, cells capable of expressing the natural recombinant AT III could be obtained by the same method with the use of pKCR5H3BAd shown in Example 6.

Some of these expression cells were transinoculated into a medium containing 1000 nM of MTX and further incubated. Some of clones incubated in the medium containing 1000 nM of MTX, which showed high expression yields, were cloned by the limiting dilution method with the use of a 96-well plate.

The expression yields of typical examples of the clones thus obtained were shown in Table 10.

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Table 10

		Expre	ssion of AT III mutant by BHK cell	
5	Mutant	Clone	Amount of secretion into medium (µg/ml)	MTX concn. (nM)
	natural recombinant AT III	F242	15-20	1000
	1R	5-41	25-30	1000
	5R	D153	13-15	1000
10	7R	3-153	20-25	1000
70	1G5R	11-1	10	1000
	6R'	5-21	15	1000
	30R	6-18	19	1000
	2G5R	6-5	16	250
15	25R	4-2	20	250
/5	35R	42-5	22	250
	29R	22-8	17	250
	2G30R	16	19	250
	7G5R	1	12	250

The amount of secretion into the medium was expressed in the mutant concentration 24 hours after replacing the medium in a state of confluent growth of cells (the amount of medium was 0.3 ml/cm²).

#### Example 8

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Incubation of mutant expression cells and purification of mutant

The AT III mutant expression cells obtained in Example 7 were incubated in a roller bottle (1750 cm²). As a medium, Dulbecco's modified Eagle medium containing 5% of fetal calf serum and MTX (final concentration being 250 nM or 1000 nM) was used. The cells were inoculated into 300 mI of the medium and incubated at 37 °C. From 3 to 4 days after the initiation of the incubation, the medium was replaced by the same amount of a fresh one everyday and the culture supernatants were combined.

The AT III mutants were purified by affinity chromatography with the use of an antibody column wherein anti AT III monoclonal antibody was bound to a support. Namely, the above-mentioned culture supernatant was charged into an antibody column which had been equilibrated with 50 mM Tris-HCl buffer pH 7.5 - 0.5 M NaCl. After washing with the same buffer, it was eluted with 0.2 M glycine-HCl buffer (pH 2.5). The eluted fractions were immediately neutralized with 1/2 times by volume as much 1 M Tris-HCl (pH 8.0). The fractions thus obtained were dialyzed against Dulbecco's PBS (-), ultrafiltrated and then used in the subsequent test. In the cases of some mutants, the eluted fractions from the antibody column was ultrafiltrated, charged into Sepharcryl S-200 and eluted with Dulbecco's PBS (-) (gel-filtration). The active fraction thus obtained was concentrated and then used in the subsequent test.

During the process of incubation and purification, each AT III mutant was determined by the EIA method with the use of anti AT III antibody.

The natural recombinant AT III employed as a control was also incubated and purified by the same method.

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## SEQUENCE LISTING

5	(1) GENERAL INFORMATION:
	(i) APPLICANT:
	(A) NAME: Eisai Co., Ltd.
10	(B) STREET: 6-10. Koishikawa 4-chome. Bunkyo-ku
	(C) CITY: Tokyo
	(E) COUNTRY: Japan
75	(F) POSTAL CODE (ZIP): 112
	(ii) TITLE OF INVENTION: HUMAN ANTITHROMBIN III MUTANTS
20	(iii) NUMBER OF SEQUENCES: 2
	(iv) COMPUTER READABLE FORM:
25	(A) MEDIUM TYPE: Floppy disk
	(B) COMPUTER: IBM PC compatible
	(C) OPERATING SYSTEM: PC-DOS/MS-DOS
30	(D) SOFTWARE: Patentin Release #1.0, Version #1.25 (EPO)
	(vi) PRIOR APPLICATION DATA:
35	(A) APPLICATION NUMBER: JP 90488/92
35	(B) FILING DATE: 10-Apr-1992
	(vi) PRIOR APPLICATION DATA:
<b>‡</b> 0	(A) APPLICATION NUMBER: JP 31855/93

(B) FILING DATE: 22-Feb-1993

45

55

	(2) INFORMATION FOR SEQ ID NO: 1:	
5	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 1395 base pairs	
	(B) TYPE: nucleic acid	
10	(C) STRANDEDNESS: double	
	(D) TOPOLOGY: linear	
15	(ii) MOLECULE TYPE: cDNA	
	(vi) ORIGINAL SOURCE:	
	(A) ORGANISM: Homo sapiens	
20		
	(ix) FEATURE:	•
	(A) NAME/KEY: CDS	
25	(B) LOCATION: 11395	
	(ix) FEATURE:	
3 <i>0</i>	(A) NAME/KEY: sig_ peptide	
00	(B) LOCATION: 196	
	(ix) FEATURE:	
35	(A) NAME/KEY: mat_ peptide	
	(B) LOCATION: 971395	
40	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:	
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15	Met Tyr Ser Asn Val lle Gly Thr Val Thr Ser Gly Lys Arg Lys Val	40
.5	-32 $-30$ $-25$ $-20$	
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	TAT	T CT	TTO	G TC	TT(	CTC	CT(	CATI	GGG	TT	C TG	G GA	C TG	C GT	G A	CC 1	rgt	9
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	CA (	C GGG	AGC	CC1	GTG	GAC	ATO	TGC	ACA	GC	C AA	G CC	CG	G GA	C AT	T (	ccc	14
	His	Gly	Ser	Pro	Val	Asp	116	: Cys	Thr	Ala	a Ly:	s Pro	Ar:	g As	p []	e F	, LO	
10	1				5					10	)				1	5		
	ATG	AAT	CCC	ATG	TGC	ATT	TAC	CGC	TCC	CCC	GAC	G AAC	A A (	G GC	A AC	T G	AG	192
	Met	Asn	Рго	Met	Cys	lle	Tyr	Arg	Ser	Pro	Gli	ı Lys	Lys	Ala	a Th	гG	lu	
15				20					25					3 (	)			
	GAT	GAG	GGC	TCA	GAA	CAA	AAG	ATC	CCG	GAG	GCC	CACC	A A C	CGC	G CG	T G	TC	240
	Asp	Glu	Gly	Ser	Glu	Gln	Lys	lle	Pro	Glu	Ala	Thr	Asn	Arg	g Ar	g V	a l	
			35					40					4 5					
20	TGG	GAA	CTG	TCC	AAG	GCC	AAT	TCC	CGC	TTT	GCT	ACC	A CT	TTC	TA.	r C	AG	288
	Trp	Glu	Leu	Ser	Lys	Ala	Asn	Ser	Arg	Phe	Ala	Thr	Thr	Phe	Ty	r G	l n	
		50					5 5					60						
25	CAC	CTG	GCA	GAT	TCC	AAG	TAA	GAC	AAT	GAT	AAC	ATT	TTC	CTG	TCA	A CO	CC	336
	His	Leu	Ala	Лsр	Ser	Lys	Asn	Asp	Asn	Asp	Asn	lle	Phe	Leu	Ser	. P.	ro	
	65					70					75					8	30	
30	CTG	AGT	ATC	TCC	ACG	GCT	TTT	GCT	ATG	ACC	AAG	CTG	GGT	GCC	TGT	` AA	T	384
,,,	Leu	Ser	lle	Ser	Thr	Ala	Phe	Ala	Met	Thr	Lys	Leu	G1y	Ala	Cys	As	in	
•					85					90					95			
	GAC	ACC	CTC	CAG	CAA	CTG	ATG	GAG	GTA	TTT	AAG	TTT	GAC	ACC	ATA	TC	T	432
35	Asp	Thr	Leu	Gln	Gln	Leu	Met	G]u	Val	Phe	Lys.	Phe	Asp	Thr	lle	Se	r	
				100		•			105					110				
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	Glu	Lys	Thr	Ser .	Asp (	Gln	lle	His I	he:	Phe	Phe	Ala	Lys	Leu	Asn	Су	S	
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	CGA	CTC	TAT	CGA A	AAA (	GCC A	AAC /	AAA 7	rcc 1	rcc .	AAG	TTA	GTA	TCA	GCC	AA	Т	528
!5	Arg.	Leu '	Tyr	Arg l	Lys 1	Ala A	Asn l	.ys S	Ser S	Ser :	Lys	Leu	Val	Ser	Ala	Asi	n	
		130				1	35					140						

	CG	с ст	T TT	T GG	A GA	C AAA	A TC	с ст	T AC	с тт	C AA	T GA	G A	CC TA	AC (	CAG	GAC	576
	Ar	g Le	u Ph	e Gl	y As	p Lys	s Se	гLе	u Th	r Ph	e As	n Gl	u Ti	ır T;	yr (	Hn	Asp	
5	14					150					15						160	
	AT	C AG	T GA	G TT	G GT	TAT A	GG.	4 GC	C AA	G CT	C CA	G CC	C C1	rg g/	AC T	тс	AAG	624
	11	e Se	r Gl	u Lei	ш Va:	l Tyr	Gly	/ Ala	a Ly:	s Le	u Gl	n Pr	o Le	eu As	sp P	he	Lys	
10					163					17						75		
	GA.	A AA	T GC	A GAO	G CAA	TCC	AGA	GCC	GCC	CAT	C AA	C AA	A TG	G GT	G T	CC	AAT	672
	G 1	u Ası	n Ala	a Glu	Glr	Ser	Arg	Ala	a Ala	a Ile	e Ası	ı Ly	s Tr	p Va	1 S	er	Asn	
15				180	)				185	5				19	0			
	AA	G ACC	G GAA	A GGC	CGA	ATC	ACC	GAT	` GTC	AT1	CC(	CTC	G GA	A GC	C A	TC	AAT	720
	Lys	s Thi	Glu	Gly	Arg	lle	Thr	Asp	Val	He	e Pro	Se	r Gl	u Al	a I	le	Asn	
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	Glu	! Leu	Thr	Yal	Leu	Val	Leu	Val	Asn	Thr	lle	Туг	Ph	e Ly	s G	lу	Leu	
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3 <i>0</i>	AAG	GCT	GAT	GGA	GAG	TCG	TGT	TCA	GCA	TCT	ATG	۸TG	T۸C	CAC	G GA	A (	GGC	864
	Lys	Ala	Asp	Gly	Glu	Ser	Cys	Ser	Ala	Ser	Met	Met	Tyr	Glr	ı Gl	u (	Gly	
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				260					265					270				
	CCC	TTC	AAA	GGT	GAT	GAC	ATC .	ACC	ATG	GTC	CTC	ATC	TTG	ccc	AA	G C	CT	960
40	Pro			Gly	Asp	Asp	lle	Thr	Met	Yal	Leu	Пe	Leu	Pro	Lys	s P	ro	
			275					280					285					
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<b>45</b>	Glu	Lys	Ser	Leu	Ala			Glu	Lys	Glu	Leu	Thr	Pro	Glu	Val	L	eu	
		290				2	295					300						

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5	30	5				310	0				31	5				320	
	CC	C CG	C TT	C CG	CATI	i GA(	G GAO	GGG	TT(	: AGT	TT(	G AA	GGAC	CAC	СТ	G CAA	1104
	Pro	э Ата	; Phe	e Arg	3 116	e Glu	ı Ası	Gly	Phe	Ser	Lei	ı Ly:	s Glu	Gln	Le	u Gln	
70					325	)				330	ı				33	5	
	GAC	CATO	GGC	CT1	GTC	GAT	СТО	TTC	AGC	CCT	GAA	AAC	TCC	AAA	CTO	C CCA	1152
	Asp	Met	Gly	' Leu	Val	Asp	Leu	Phe	Ser	Pro	Glu	Lys	Ser	Lys	Lei	ı Pro	
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20													AGA				1344
30	Thr	Phe	Lys	Ala	Asn	Arg	Рго	Phe	Leu	Val	Phe	lle	Arg	Glu	Val	Pro	
					405					410					415		
													CCT				1392
35	Leu	Asn			lle	Phe	Me t	Gly.	Arg	Val A	Ala.	Asn	Рго (	Cys '	Va]	Lys	
				420	•				425				4	30			
	TAA																1395

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		-15					-10					-5				•
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25	1				5					10						
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	6 5					70					75					80
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				100					105					110		
45	Glu			Ser	Asp	Gln	lle	His	Phe	Phe	Phe	Ala	Lys	Leu	Asn	Cys
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		130	)				13	5				140	)			
5	Ar	e Lei	ı Phe	Gly	' Asp	) Lys	S Se	r l.e	u Thi	r Phe	e Asr	Gli	. Th	г Туг	Gli	n Asp
	14	5				150	)				155	•				160
	ile	e Ser	Glu	lei	(Va)	Тут	Gla	r Ala	a Lys	Lei	ı Glr	Pro	Lei	ı Asp	Phe	Lys
					165	1				170	)				175	•
10	Glı	ı Asn	Ala	Glu	Gln	Ser	Arg	: Ala	a Ala	lle	: Asn	Lys	Tr	Val	Ser	Ásn
				180					185			•		190		
	Lys	Thr	Glu	Gly	Arg	He	Thr	Asp	Val	Пe	Pro	Ser	Glu	Ala	He	Asn
15			195					200	)				205			
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	225					230					235					240
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				250				•	265					270		-
	Pro	Phe		Gly	Asp	Asp	lle	Thr	Met	Va l	Leu	lle	Leu	Pro	Lys	Pro
30			275					280					285	٠		
•	Glu	Lys	Ser	Leu	Ala	Lys	Val	Glu	Lys	Glu	Leu	Thr	Pro	Glu	۷al	Leu
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	305					310					315					320
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	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
5	Ser 385	Thr	Ala	Val	Yal	11e 390	Ala	Gly	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val
10					405		Pro			410					415	
	Leu	asn	inr	420	ne.	rne	Met	αįγ	425	Val	Ala	Asn	Pro	430	Val	Lys
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(1) GENERAL INFORMATION:

## SEQUENCE LISTING

_	(4)	
5 10	<ul> <li>(i) APPLICANT:</li> <li>(A) NAME: Eisei Co., Ltd.</li> <li>(B) STREET: 6-10, Koishikawa 4-chome, Bunkyo-ku</li> <li>(C) CITY: Tokyo</li> <li>(E) COUNTRY: Japan</li> <li>(F) POSTAL CODE (2IP): 112</li> </ul>	
	(ii) TITLE OF INVENTION: Human Antithrombin III Mutants	
	(iii) NUMBER OF SEQUENCES: 81	
15	(iv) COMPUTER READABLE FORM:  (A) MEDIUM TYPE: Floppy disk  (B) COMPUTER: IBM PC compatible  (C) OPERATING SYSTEM: PC-DOS/MS-DOS  (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)	
20	<pre>(vi) PRIOR APPLICATION DATA:     (A) APPLICATION NUMBER: JP 90488/92     (B) FILING DATE: 10-Apr-1992     (A) APPLICATION NUMBER: JP 31855/93     (B) FILING DATE: 22-Feb-1993</pre>	
25	(2) INFORMATION FOR SEQ ID NO: 1:	
30	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 1395 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
35	(vi) ORIGINAL SOURCE:  (A) ORGANISM: Homo sapiens	
	(ix) FEATURE:  (A) NAME/KEY: CDS  (B) LOCATION: 11395	
40	(ix) FEATURE: (A) NAME/KEY: sig_peptide (B) LOCATION: 196	
45	(ix) FEATURE:  (A) NAME/KEY: mat_peptide  (B) LOCATION: 971395	
	() CHOUPING DESCRIPTION, SEC IT NO. 1-	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:	
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5	Hi:	C GGG S Gl	G AGO y Se:	C CCI	GTO Val	GAC L Asp	ATC	TGC Cys	ACA Thi	A GCC Ala 10	a Lys	G CCC	G CGC	GA(	C AT	r ccc Pro	144
10	AT(	G AA! t Asi	r cco	ATO Met	Cys	: ATT	TAC	CGC Arg	TCC Ser 25	Pro	GAC Glu	AAC Lys	AAC Lys	GCX Ala 30	Th	GAG Glu	192
	GA] Asp	GAC Glu	GGC 1 Gly 35	, Ser	GAA Glu	CAA Gln	AAG Lys	ATC Ile 40	Pro	GAG Glu	GCC Ala	ACC Thr	: AAC : Asn 45	Arç	G CGT	GTC Val	240
15	TGC Trp	GAA Glu 50	Let	TCC Ser	AAG Lys	GCC Ala	AAT Asn 55	TCC Ser	CGC	TTT Phe	GCT Ala	ACC Thr 60	Thr	TTC	TAI Tyr	CAG Gln	288
20	CAC His 65	Leu	GCA Ala	GAT Asp	TCC Ser	AAG Lys 70	AAT Asn	GAC Asp	AAT Asn	GAT Asp	AAC Asn 75	ATT Ile	TTC Phe	CTG Leu	TCA Ser	CCC Pro 80	336
	CTG Leu	AGT Ser	ATC Ile	TCC Ser	ACG Thr 85	GCT Ala	TTT Phe	GCT Ala	ATG Met	ACC Thr 90	Lys	CTG Leu	GGT Gly	GCC Ala	TGT Cys 95	Asn	384
25	GAC Asp	ACC Thr	CTC Leu	CAG Gln 100	CAA Gln	CTG Leu	ATG Met	GAG Glu	GTA Val 105	TTT Phe	AAG Lys	TTT Phe	GAC Asp	ACC Thr 110	IJ.e	TCT	432
	GAG Glu	AAA Lys	ACA Thr 115	TCT Ser	GAT Asp	CAG Gln	ATC Ile	CAC His 120	TTC Phe	TTC Phe	TTT Phe	GCC Ala	AAA Lys 125	CTG Leu	AAC Asn	TGC Cys	480
30	CGA Arg	CTC Leu 130	TAT Tyr	CGA Arg	AAA Lys	GCC Ala	AAC Asn 135	AAA Lys	TCC Ser	TCC Ser	AAG Lys	TTA Leu 140	GTA Val	TCA Ser	GCC Ala	AAT Asn	528
35	CGC Arg 145	CTT Leu	TTT Phe	GGA Gly	GAC Asp	AAA Lys 150	TCC Ser	CTT Leu	ACC Thr	TTC Phe	AAT Asn 155	GAG Glu	ACC Thr	TAC Tyr	CAG Gln	GAC Asp 160	576
	ATC Ile	AGT Ser	GAG Glu	TTG Leu	GTA Val 165	TAT Tyr	GGA Gly	GCC Ala	AAG Lys	CTC Leu 170	CAG Gln	CCC Pro	CTG Leu	GAC Asp	TTC Phe 175	AAG Lys	624
40	GAA Glu	AAT Asn	Ala	GAG Glu 180	Gln	TCC . Ser .	AGA Arg	Ala	GCC Ala 185	ATC Ile	AAC Asn	AAA Lys	TGG Trp	GTG Val 190	TCC Ser	AAT Asn	672
45	AAG Lys	ACC Thr	GAA Glu 195	GGC Gly	CGA Arg	ATC /	Thr .	GAT Asp 200	GTC Val	ATT Ile	Pro Pro	Ser	GAA Glu 205	GCC Ala	ATC Ile	AAT Asn	720
	Glu	CTC Leu 210	ACT Thr	GTT   Val	CTG Leu	GTG ( Val I	CTG ( Leu \ 215	GTT . Val .	AAC . Asn	ACC . Thr	Ile '	TAC Tyr 220	TTC . Phe	AAG Lys	GGC Gly	CTG Leu	768
50	TGG . Trp 225	AAG Lys	TCA . Ser	AAG : Lys !	Phe :	AGC C Ser P 230	CT (	SAG /	AAC A Asn '	Thr 1	AGG A Arg 1 235	AAG ( Lys (	GAA ( Glu l	CTG Leu	Phe	TAC Tyr 240	816

	Ly.	G GC s Al	T GAS	T 662 p 613	A GA0 y Glu 245	ı Seı	TGT Cys	TC.	t GC r Al	A TC a Se 25	r Me	G AT t Me	G TA T Ty	C CA r Gl:	G GA r. Gl 25	A GGC u Gly S	864
5	Ly:	G TT s Ph	C CG e Arq	TAT 177 260	: Arg	G CGC g Arg	GTG Val	GC: Ala	F GA. e Gl: 26:	u G1;	C ACC y Th.	c ca r Gl	G GT( n Val	S CT: 1 Leu 270	u G1	G TTG u Leu	912
10	Pro	TTO Pho	C AAA E Lys 275	: CJ?	GAT Asp	GAC Asp	ATC Ile	AC0 Thi 280	Me	G GT(	C CTO	C ATO	C TT( = Let 285	Pro	C AAO D Lys	G CCT s Pro	960
	GAG Glu	E AAC 1 Lys 290	s Ser	CTG Leu	GCC Ala	AAG Lys	GTT Væl 295	GAG Glu	Lys	GAZ Glu	A CTO	AC0 Th: 300	Pro	GAA Glu	GT(	CTG Leu	1008
15	CAG Gln 305	Glu	TGG Trp	CTG Leu	GAT Asp	GAA Glu 310	TTG Leu	GAG Glu	GAG Glu	ATG Met	ATG Met 315	Leu	GTG Val	GTC Val	CAC His	ATG Met 320	1056
20	CCC Pro	CGC Arg	TTC Phe	CGC Arg	ATT Ile 325	GAG Glu	GAC Asp	GGC GGC	TTC Phe	AGT Ser 330	Leu	AAG Lys	GAG Glu	CAG Gln	CTG Leu 335	CA.A. Gln	1104
	GAC Asp	ATG Met	GGC	CTT Leu 340	GTC Val	GAT Asp	CTG Leu	TTC Phe	AGC Ser 345	CCT Pro	GAA Glu	AAG Lys	TCC Ser	AAA Lys 350	CTC Leu	CCA Pro	1152.
25	GGT Gly	ATT Ile	GTT Val 355	GCA Ala	GAA Glu	GGC Gly	Arg	GAT Asp 360	GAC Asp	CTC Leu	TAT Tyr	GTC Val	TCA Ser 365	GAT Asp	GCA Ala	TTC Phe	1200
30	CAT His	AAG Lys 370	GCA Ala	TTT Phe	CTT Leu	GAG Glu	GTA . Val . 375	AAC Asn	GAA Glu	GAA Glu	GGC Gly	AGT Ser 380	GAA Glu	GCA Ala	GCT Ala	GCA Ala	1248
	AGT Ser 385	ACC Thr	GCT Ala	GTT Val	Val	ATT Ile 1 390	GCT (	SGC Sly	CGT Arg	TCG Ser	CTA Leu 395	AAC Asn	CCC Pro	AAC Asn	AGG Arg	GTG Val 400	1296
35	ACT Thr	TTC Phe	AAG Lys	Ala.	AAC . Asn . 405	AGG ( Arg !	CCT :	TTC Phe	CTG Leu	GTT Val 410	TTT Phe	ATA Ile	AGA Arg	Glu	GTT Val 415	CCT Pro	1344
40	CTG Leu	AAC Asn	Thr .	ATT : Ile : 420	ATC :	TTC A Phe N	ATG G	ly :	AGA Arg 125	GTA   Val 1	GCC : Ala :	AAC Asn	Pro (	TGT ( Cys \ 430	GTT . Val	AAG Lys	1392
	TAA				•												1395

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 464 amino acids
  (B) TYPE: amino acid
  (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

50

			(×:	i) S	EQUE	NCE :	DESC	RIPT:	ION:	SEQ	ID I	۷O: ۵	2:				
		Me1 -33	ту: 2	-30	r Ası	n Va.	1 I2e	e Gly	7 Th: -2	r Vai	l Th	s Se	r Gl	y Ly:		Ly:	s Val
5		Тул	Let -15	Le	ı Sei	: Le	ı Lev	Le:	11e	∈ Gly	y Phe	rri	Ası !-		Va]	l Th	c Cys
		His I	Gly	/ Se	r Pro	Val	l Asp	o Ile	e Cys	Th:	: Ala		s Pro	o Arg	Asp	> Ile	e Pro
10		Met	Asr	Pro	Met 20	Cys	s Ile	. Tyr	Arç	ser 25		Glu	Lys	s Lys	: Ala		c Glu
		Asp	Glu	35 35	/ Ser	Glu	ı Glr	Lys	1 <u>1</u> e		Glu	Ala	Thi	Asn 45		Arç	y Val
15		Trp	Glu 50	Lei	Ser	Lys	. Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60		Phe	туг	Gln
		His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75		: Fhe	Leu	Ser	Pro 80
20		Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90		Leu	Gly	Ala	Cys 95	Asn
		Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105		Lys	Phe	Asp	Thr 110		ser
25		Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120		Phe	Phe	Ala	Lys 125		Asn	Cys
		Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140		Ser	Ala	Asn
30	** *	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
		Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
35		Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn
		Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
40		Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
		Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
45		Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
		Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270	Glu	Leu
50		Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu		Leu 285	Pro	Lys	Pro

	GII	380 1 FAS		. Leu	. Ala	Ly:	s Va. 25.		r F2.8	s Glu	l Let	30 30		o G1	ט עו	≥l L	÷
5	G1:	. Glu	Trp	Leu	Asp	. Gl: 310		n CJ:	o Glu	) Met	Met 315		u Va	l Vá	:1 H:	is Me 33	
	Pro	Arg	Phe	Arg	11e 325		ı Asp	o Gly	/ Phe	Ser 330		Lys	E 61	υ Gl	n Le 33		L
	Asp	Met.	GJ 'n	Leu 340	Va]	Asp	Leu	Phe	Ser 345	Pro	Glu	ŗ7.	S Se	r Ly 35		u Pr	٠,
10	G] y	lle	Val 355	Æla	Glu	GJ 7	Arg	360		Leu	Tyr	٧al	Se:		₽ ⊁l	a Ph	: 6
	His	Lys 370	Ala	Phe	Leu	G] u	Val 375		Glu	Glu	Gly	Ser 380		a Al	a Al	a Al	ē
<b>15</b>	Ser 385	Thr	Ala	Val	Val	11e 390	Ala	Gly	Arg	Ser	Leu 395	Asn	Pro	As:	n Ar	g Va 40	
	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Ph∈	Leu	Val 410	Phe	Il∈	Arg	G11	) Va 41		c
20	Leu	Asn	Thr	11e 420	Ile	Phe	Met	ely	Arg 425	Vāl	Ala	Asn	Pro	Cys 430		l Lys	5
25	(2)	1NF0	i) S (A (B	EQUE ) LE ) TY	NCE NGTH PE:	CHAF : 46 amir	act:	ERIST mino cid	3: FICS: acid								
80		(ii)							EO T	D NO	. 3.						
	Met -32	Tyr :										Gly	Lys -20	Arg	Lys	Val	
	Tyr	Leu 1 -15	Leu S	Ser 1	Leu :		Leu -10	Ile	G1 y	Phe 1	Trp A	-5	C7.2	Val	Thr	C}'s	
	His 1	Gly s	Ser :	Pro N	/al /	asp	Ile	Cys	Thr A	Ala 1 10	ys F	Pro .	Arg	Asp	11e 15	Pro	
10	Met J	Asn E	Pro l	1et 0 20	ys I	île	Tyr .	Arg	Ser I 25	Pro G	1u 1	ys :	Lys	Ala 30	Thr	Glu	
٠.	Asp (	Glu G	Sly S 35	Ger G	Slu C	Sln :	Lys :	11e 40	Pro 0	Slu A	la T	'nr i	4.5 4.5	Arg	Arg	Va <u>1</u>	
15	Trp (	Glu I 50	Jeu S	er 1	ys ?	la :	55 55	Ser i	Arg F	he A		hr (	Thr	Phe	Tyr	Gln	
	His I 65	Leu A	la A	sp S	er l	ys <i>!</i> 70	Asn A	Asp A	Asn A		sn I 75	le E	Phe	Leu	Ser	Pro EO	
0	Leu S	Ser I	le S		hr A 85	la E	he A	la Þ		hr L 50	√s Ŀ	eu (	31 y 3	4la	Cy's 95	Asn	

	As	p Th	r Le	υ Gl 10	n Gl: O	n Le	u Me	t G]	lu Va	al Pi 05	ne L	ys P	he A		hr I. 10	le S∈
5	G1	u Ly	s Th 11	: Se. 5	r Ası	p Gl	n Il	e Hi 12	s P}	ne Ph	ne Pl	ne A.	la L	ys Le 25	eu A:	sn Cy
	Ar	g Le 13	и Ту О	r Ar	g Ly:	s Al	a As 13	n Ly 5	s Se	er Se	er L		eu Va 40	al Se	er Al	la Ası
10	Ar 14	g Le <sup>.</sup> 5	u Ph	e Gly	y Asp	15	s Se O	r Le	u Tr	ir Ph	e As	n G]	lu Th	ar Ty	yr Gl	n Ası 160
	11	e Se	r Gl	u Leu	va) 165	Ту.	r Gl	y Al	a Ly	's Le 17	u G1	n Pi	o Le	eu As	sp Ph 17	e Lys
15	Gl	u Ası	n Ala	a Glu 180	ı Gln	Se:	r Ar	g Al	a Al 18	a Il 5	e As	n Ly	's Ti	p Va		r Asr
	Lys	5 Thi	Gl: 195	u Gly 5	' Arg	Ile	≘ Thi	20	p Va O	1 11	e Pr	o Se	r Gl 20		a Il	e Asn
20	Glı	1 Leu 210	Thi	: Val	Ъеи	Val	Let 215	va:	l As	n Th	r Il	е Ту 22		e Ly	s Gl	у Leu
	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	ı As:	n Th	r Ar 23	g Ly 5	s Gl	u Le	u Ph	e Tyr 240
25	Lys	: Ala	Asp	Gly	Glu 245	Ser	Cys	Sei	Al.	a Se: 250	r Me O	t Me	t Ty	r Gl	n G1 25	u Gly 5
	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Alā	26!	u Gly S	y Th	r Gl	n Va	1 Le 27		u Leu
30	Pro	Phe	Lys 275	Gly	Asp	A.sp	Ile	Thr 280	Met	. Val	Lei	ı Il	e Le: 28:		o Ly:	s Pro
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	ı Leı	Th:		o Glu	ı Val	l Leu
35	303					310					315	•				Met 320
					323					330					335	
40	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350		Pro
-	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365		Ala	Phe
45	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
<b>,</b>	Ser 385	Thr	Ala	Val	Val :	Ile 390	Ala	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
					405					410					415	
50	Leu .	Asn '	Th r	11e : 420	Ile E	Phe 1	Met	Gly	Arg 425	Val	Ala	Asn	Pro	Cys	Val	Lys

(2) INFORMATION FOR SEQ ID NO: 4:

5			(i)	(A) (B)	LENG TYPE	5ТН: С: ал	lARAC 464 nino ': li	amir acio	ic ac							
		(i)	i) M	OLEC	ULE	TYPE	: pr	otei	n							
		(x)	i) S	ΕΟΝΕ	NCE	DESC	FIPT	: 4O I	SEQ	ID	ю:	4:				
	Me: -3:		s Se -3		n Va	l I1	e Gl	у Тr. -2		l Th	r Se	r Gl	у Ly -2		g Ly	's Va
15	ту	-15	ı Lei	u Se.	r Le	u Le	u Le		e G1	y Ph	e Tr	p As		s Vā	l Th	r Cy:
.5		el7	) Se	r Pro		l As	p Il	в Су	s Th.	r Al		s Pr	o Ar	g As		e Pro 5
9	Met	Asn	Pro	: Μετ 20	Cys	s Il	е Туз	r Ar	g Sei 25		o Gj	n Fys	s Ly:	s Ala 30		r Glı
	Asp	Glu	35 Gl }		: Gli	ı Clı	n Lys	11e		Glı	ı Ala	a Thi	4.5 4.5	_	j Ar	g Val
5	Trp	Glu 50	Leu	Ser	Lys	s Ala	Asn 55	Ser	Arg	Phe	÷ Ala	Thr 60		: Phe	ту:	r Gln
	His 65	Leu	Ala	Asp	Ser	7 Lys		Asp	Asn	Asp	Asr 75		: Phe	: Leu	s Se	Pro 80
0	Leu	Ser	Il€	Ser	Thr 85	Ala	Phe	A.la	Met	Thr 90		Leu	GJ Y	' Ala	Су <b>з</b> 95	Asn
	Asp	Thr	Leu	Gln 100		Leu	Met /	Glu	Val 105	Phe	Lys	Ph∈	Asp	Thr 110		: Ser
5	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125		Asn	Cys
	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
o	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Туг	Glγ	Ala	Lys	Leu 170	Gln	Pro		Asp		
5	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn
			195				Thr	200					205			
)	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	lle,	Туг 220	Phe	Lys	Gly	Leu .

		Trp 225	o Lys	Ser	Lys	Phe	Se 1 230	r Pro	Glu	Asn	Thr	Arg 235	Lys	s Glu	Leu	Phe	Tyr 240
5		Lys	s Ala	Asp	Gly	Glu 245	Se:	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
		ГÀЗ	Phe	Arg	Туг 260	Arg	Arg	y Val	Ala	G1 ນ 265	Gly	Thr	Gln	Val	Leu 270		Leu
10		Pro	Phe	Lys 275	Gly	Asp	Asp	lle	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
			230					295					300				Leu
15		303					310			Glu		315					320
						323				Phe	330					335	
20					340					Ser 345					350		
				223					360	Asp				365			
25			370					3/5		Glu			380				
		303					390			Arg		395					400
30						405				Leu	410					415	
		Leu	Asn	Thr	11e 420	Ile	Phe	Met	Gly.	Arg 425	Val ,	Ala .	Asn		Cys 430	Val	Lys
35		(2)	INFO	i) S! (A) (B)	EQUEI LEI TYI	NCE ( NGTH PE: a	CHAR : 46 amin	ACTE	id	: ICS: əcid:	5						
40								prote									
	<u> </u>	let :	ryr s	er A						EQ II Val T				Lys A	rg L	vs \	/al
45		yr I		30			eu L	-	25	ily P			-	-20			
	н	is G	sly s	er P	ro V	al A 5	sp I	le C	ys T	hr A	la L	ys P	-	rg A		le P 15	ro
50	М	et A	sn P	ro M	et C	ys I	le T	yr A	rg S	er P 25	ro G	lu L	ys L				lu

		Asj	p Glu	1 Gl <sub>3</sub>		r Gl	u Gl	n Ly		e Pr O	o 61	u Al	a Th		n Ar 5	g Ar	g Val
5		Try	5 Glu 50		ı Sei	c Ly.	£ Al	a As: 5:		r Ar	g Ph	e Al	a Th é		r Ph	e T;	r Gln
J		Ні s 6.5		1 <u>7-1</u> a	e Asp	Se.	r Ly. 7		n As	p As	n As	p Asi 7		e Ph	e Le	u Se	r Pro 80
10		Lei	ser	116	s Ser	Th:		a Phe	e Ala	a Me	t Th S		s Lei	u Gl	y Al.	ā С;' 9	s Asn 5
70		Asp	Thr	Leu	100		i Lei	u Met	: Glı	u Va. 10:		≞ Lys	: Phe	÷ Æsj	7h:		e Ser
		G1 u	Lys	Thr 115		Asp	Glr	n Ile	His 120		∈ Phe	≥ Phe	: Ala	Ly:		ı Ası	n Cys
15		Arg	Leu 130		Arg	Lys	Ala	Asn 135		S S e s	r Sei	: Lys	140		Sei	: Ala	a Asn
		Arg 145		Phe	Gly	Asp	Lys 150	Ser	Leu	Th:	? Phe	Asn 155		Thr		Glr	160
20		Ile	Ser	Glu	Leu	Val 165		ej A	Ala	Lys	170		Pro	Leu	Asp	Phe 175	Lys
		Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185		Asn	Lys	Trp	Val 190		Asn
25		Lys	Thr	Glu 195	Gly	Arg	Il€	Thr	Asp 200		Ile	Pro	Ser	Glu 205		Ile	Asn
		Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Il∈	Tyr 220	Phe	Lys	GJy	Leu
30		Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Туг 240
		Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
<b>35</b>		Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Gl u 265	СŢУ	Thr	Gln	Va <u>l</u>	Leu 270	Glu	Leu
		Pro		Lys 275	Gly	A.sp	Asp	Ile	Thr 280	Met	Val	Leu	lle	Leu 285	Pro	Lys	Pro
40			290					Val 295					300				
		305					310	Leu				315					320
45						325		Asp			330					335	-
					340			Leu		345					350		
50	,	Gly		/al . 355	Ala (	Glu	Gly.	Arg .	Asp 360	Asp	Leu	Tyr '		Ser. 365	Asp.	Ala	Phe

	Hi	s Ly:	s Ala	a Ph	e Le	u Gli	u Va 37	l As S	n Gl	u Gli	u G1;	y Se 38		u Al	a Al	a Ala
5	5e. 38.	r Thi	r Ala	a Val	l Va	1 Ile 390	≥ Il	e Pr	o Ar	g Se	r Let 39:		n Pr	o As	n Ar	g Val 400
	Th.	r Phe	≥ Ly:	s Ala	a Ası 40	n Arç 5	Pr:	o Ph	e Le	บ Val 41(		≥ Il	e Ar	g Gl	u Val 41:	
70	Lei	ı Ası	Thi	11e 420	e Ile O	e Phe	e Me	c Gl	y Ar 42	g Val	Ala	a Ası	n Pro	O Cy:		Lys
	(2)	INE	ORM	AO ITA	V FOF	R SEQ	) ID	ио:	6:							
15			(	(A) I	ENGT	E CHA TH: 4 : ami LOGY:	64 a	amino acid								
		(ii	.) MC	LECU	JLE T	YPE:	pro	cteir	ו							
20										ID N						
	Met -32	Tyr	Ser -30	Asn	ı Val	Ile	G1?	7 Thr		Thr	Ser	G1	-20		i Lys	Val
25	Tyr	-15	Leu	Ser	Leu	. Leu	Leu ~10	ı Ile	e G1 y	/ Phe	Trp	A.s.p - 5		val	Thr	Cys
	His 1	Gly	Ser	Pro	Val 5	Asp	Ile	Cys	Thi	: Ala 10		Pro	Arg	Asp	Ile 15	
30	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Arg	Ser 25	Pro	Glu	Lys	Lys	Ala 30		Glu
	Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	Ile 40		Glu	Ala	Thr	Asn 45		Arg	Val
35	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60	Thr	Phe	Туг	Gln
	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
40	Leu	Ser	Ile	Ser	Th: 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gĺn	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
45	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
50	Ile	Ser	Glu	Leu	Val 165	Tyr	Gl y	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys

	Gin	u Ası	n Ala	: Glu		ı Se.	r Ar	g Al	= Ala 18:		e Asi	) Ly:	s Trj	5 Va.		r Asn
5	Lys	s Thi	r Glu 195		· Arg	: I)	e Thi	r Asy 200		1 116	e Pro	Ser	61 c 205		≥ Il	e Asn
	Glu	Le: 210		Val	Leu	Val	Lei 219		l Asr	Th:	: I1e	Tyr 220		· Lys	s Gl	y Leu
10	Trp 225	Lys	s Ser	Lys	Phe	Ser 230		Glu	Asn	Thr	Arg 235		Glu	Let	Ph:	= Tyr 240
	Lys	Ala	Asp	GJ À	Glu 245	Ser	Cys	Ser	₽.1 ē	Ser 250		Met	Tyr	Gln	Glu 255	Gly
15	Lys	Phe	. Arg	Tyr 260	Arg	Arg	Val	A.la	61 u 265		Thr	Gln	Val	Leu 270		Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280		Val	Leu	Ile	Leu 285	Pro	Lys	Pro
20	Glu	Lys 290		Leu	Дlа	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
25	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	.Glu	Gln	Leu 335	Gln
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Ph∈	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
30	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu		Ser 380	Glu	Ala	Ala	Ala
35	Ser 385	Thr	Ala	Val	Val	11e 390	Gly	Pro	Arg		Leu . 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys :		Asn . 405	4.rg	Pro	Phe		Val 410	Phe :	Ile .	Arg		Val 415	Pro
. 40	Leu	Asn	Thr :	Ile : 420	Ile 1	Phe .	Met		Arg 1 425	Val :	Ala /	Asn :		Cys 430	Val	Lys
	(2)	INFO	PMAT	I NOI	FOR S	SEQ :	ID N	0: 7	:							
45			(B)	LE)	OLOG PE: a	464 mino	am.	ino : id		5						
50			MOLE													
		(xi)	SEQU	ENCE	DES	CRIP	TION	∛: SE	O II	NO:	7:					

	Ме - 3	t Ту 2	r Se: -30	As:	n Va.	1 11	e Gl	y Th -2	r Va 5	1 Th.	r Se	r Gl	y Ly -2		g Ly	s Val
5	ту	r Le	ı Let 5	ı Se.	r Le	n Fe	u Lei -10	u Il.	e Gl	y Phe	e Tr	P As		s Va	l Th	r Cys
	Hi	s Gly l	y Sei	r Pro	o Val	l Asy	p Ile	е Су:	s Th	r Ala		s Pr	o Ar	g As	p Il 1	e Pro 5
10	. Me	t Ası	n Pro	Met 20	Cys	5 Ile	е Туз	r Arç	3 Se. 2		Glu	ı Ly:	s Ly.	s Al.		r Glu
	As	p Gli	35 35	, Ser	: Glu	ı Glr	ı Lys	3 Ile 4(	e Pro	Glu	a Ala	Thi	4 Ası		g Ar	g Val
15	Tr	P Glu 50	Leu )	Ser	Lys	Ala	Asr 55	Ser	Arq	g Phe	: Ala	Th:		r Phe	e Ty:	r Gln
	Hi:	s Leu 5	Ala	Asp	Ser	: Lys 70	Asn	Asp	Asr	a Asp	Asr 75		Phe	e Le	ı Se	r Pro 80
20	Let	ı Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90		Leu	Gly	/ Ala	95	s Asn
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105		Lys	Phe	Asp	Th:		e Ser
25	Glı	l Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	: Phe	Phe	Ala	Lys 125		ı Asr	ı Cys
	Arg	130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140		Ser	: Ala	ı Asn
30	145	ı				150					155					160
	- •	Ser			165					170					175	_
35		Asn		180					185					190		
		Thr	195					200					205			
40		Leu 210					215					220				
	225					230					235					240
<i>1</i> 5		Ala			245					250					255	-
		Phe		260					265					270		
50		Phe	275					280					285			
	Glu	Lys 290	Ser	Leu .	Ala	Lys	Val 295	Glu	Lys	Glu :		Thr 300	Pro	Glu	Val	Leu

	305		Trp	Leu	Asp	310		. Glu	ı Gjr	Me:	315		u Val	) Val	l His	s Me 32
5	Pro	Arg	Phe	Arg	11e 325		Asp	Gly	/ Phe	Se:		ነ ይሃ።	s Gl	u Gli	n Lei 333	
	Asp	Met	GJ Y	Leu 340		Asp	Let	Ph∈	Ser 345		Glu	Lys	s Se	r Lys 350		ı Pr
10	61 y	Il∈	Val 355		61 u	<b>G</b> J 7	Arg	360	Asp	Leu	туг	Val	Se :	-	> A.1 a	Ph:
·	His	Lys 370	ΑĴā	Phe	Leu	Glu	Val 375		Glu	Glu	Gly	Se:		ı Ala	. Ala	Ala
15	Ser 385	Thr	Ala	Val	Vāl	11e 390	_	Pro	Arg	Ser	Leu 395		Pro	Asr	. Arg	400
	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	Leu	Val 410		Ile	Arg	Glu	Val 415	
20	Leu	Asn	Thr	Ile 420	Il€	Phe	Met	Gly	Arg 425	Val	Ala	Asn	Pro	Cys 430		Lys
	(2)	INFO	RMAI	rion	FOR	SEQ	ID	NO:	8:							
25		. !	( Z	_	NGTI PE:	1: 4: amin	64 as	mino cid	rics: acio							
				ECUI					. PO T	ים או	. P.					
30		(22)	ಎಲ್ಲ	10 5 14 C	E DE	JOERI	EIIC		3 T.O. 1	D M	): 8:					
	Met -32		Ser -30	Asn	Val	Ile	Gly		Val	Thr	Ser	Gly	Lys -20	Arg	Lys	Val
25	-32	_	-30				_	Thr -25					-20			
25	-32 Tyr	Leu -15	-30 Leu	Ser	Leu	Leu	Leu -10	Thr -25	Val	Phe	Trp	Asp -5	-20 Cys	Val	Thr	Cys
	Tyr His	Leu -15 Gly	-30 Leu Ser	Ser Pro	Leu Val 5	Leu Asp	Leu -10 Ile	Thr -25 Ile Cys	Val	Phe Ala 10	Trp Lys	Asp -5 Pro	-20 Cys Arg	Val Asp	Thr	Cys Pro
95 <u>.</u> 10	Tyr His	Leu -15 Gly Asn	-30 Leu Ser Pro	Ser Pro Met 20	Leu Val 5 Cys	Leu Asp Ile	Leu -10 Ile Tyr	Thr -25 Ile Cys	Val Gly Thr	Phe Ala 10 Pro	Trp Lys Glu	Asp -5 Pro	-20 Cys Arg	Val Asp Ala 30	Thr Ile 15 Thr	Cys Pro Glu
70	Tyr His 1 Met	Leu -15 Gly Asn	-30 Leu Ser Pro Gly 35	Ser Pro Met 20 Ser	Leu Val 5 Cys Glu	Leu Asp Ile Gln	Leu -10 Ile Tyr	Thr -25 Ile Cys Arg	Val Gly Thr Ser 25	Phe Ala 10 Pro Gľu	Trp Lys Glu Ala	Asp -5 Pro Lys	-20 Cys Arg Lys Asn 45	Val Asp Ala 30 Arg	Thr Ile 15 Thr	Cys Pro Glu Val
	Tyr His 1 Met Asp	Leu -15 Gly Asn Glu Glu 50	-30 Leu Ser Pro Gly 35 Leu	Ser Pro Met 20 Ser	Leu Val 5 Cys Glu Lys	Leu Asp Ile Gln Ala	Leu -10 Ile Tyr Lys Asn 55	Thr -25 Ile Cys Arg Ile 40 Ser	Val Gly Thr Ser 25	Phe Ala 10 Pro Gľu Phe	Trp Lys Glu Ala Ala	Asp -5 Pro Lys Thr	-20 Cys Arg Lys Asn 45 Thr	Val Asp Ala 30 Arg	Thr Ile 15 Thr Arg	Cys Pro Glu Val
70	Tyr His I Met Asp Trp His 65	Leu -15 Gly Asn Glu 50 Leu	-30 Leu Ser Pro Gly 35 Leu	Ser Pro Met 20 Ser Ser	Leu Val 5 Cys Glu Lys	Leu Asp Ile Gln Ala Lys 70	Leu -10 Ile Tyr Lys Asn 55	Thr -25 Ile Cys Arg Ile 40 Ser	Val Gly Thr Ser 25 Pro	Phe Ala 10 Pro Glu Phe Asp	Trp Lys Glu Ala Ala Asn 75	Asp -5 Pro Lys Thr Thr 60	-20 Cys Arg Lys Asn 45 Thr	Val Asp Ala 30 Arg Phe	Thr Ile 15 Thr Arg	Cys Pro Glu Val Gln Fro 80

	G]	lu Ly	's Th	r Se 5	r As	p Gl	n Il	.е Н: 13	is P	he P	he l	Phe	Al a	Ly.	s Le 5	eu A	sn Cys
5	Ai	g Le 13	u Ty O	r Ar	g Ly	s Al	a As	n Ly	'5 S	er S	er 1	Σys	Leu 140	Va.	l Se	r A	la Asn
	Ar 14	g Le 5	u Ph	e Gl	y As	р Ly 15	s Se O	r Le	eu Ti	hr P	he A	.sn .55	Glu	Th	ту	r G	ln Asp 160
10	11	e Se	r Gl	u Le	u Vai 16!	l Ту 5	r Gl	y Al	a L	ys L	eu 0 70	ln !	Pro	Let	ı As	p Ph 17	ne Lys 5
	Gl	u As	n Ala	a Gli 180	u Glr	n Se	r Ar	g Al	a Al 18	a I:	le A	sn l	Lys	Trp	Va.	l S∈ 0	er Asn
15	Ly	s Th.	r Glv 195	u Gly	y Arç	Į Ile	e Th.	r As 20	p Va O	1 11	le P	ro S	Ser	Glu 205		a Il	e Asn
	Glı	u Let 210	ı Thi	Val	Leu	va]	l Let 215	ı Va.	l As	n Th	er I	le I	)yr 20	Phe	Lys	s Gl	y Leu
20	Trp 225	Lys 5	s Ser	: Lys	Phe	Ser 230	Pro	Gl:	J As	n Th	r A. 2:	rg L 35	ys	Glu	Let	ı Ph	e Tyr 240
	Lys	s Ala	Asp	Gly	Glu 245	Ser	Cys	Se:	r Ala	a Se 25	r Me	et M	i∈t	Tyr	Gln	G1 25	u Gly 5
25	Lys	Phe	Arg	Туг 260	Arg	Arg	Val	Ala	Gl: 26	u Gl 5	у Т	nr G	ln	Val	Leu 270		u Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	t Va	l Le	u I		Leu 285	Pro	Lys	5 Pro
30	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	s Gl	u Le	u Ti	hr 00	Pro	Glu	Va 1	. Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	ı Met	. Ме 31	t Le 5	∍u '	Val	Val	His	Met 320
35	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Le	u Ly	's (	31 u	Gln	Leu 335	
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glı	Ly	s S		Lys 350	Leu	Pro
40	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Туг	: Va		er <i>i</i> 65		Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	G1?	38	r G O	lu A	Ala	Ala	Ala
45	Ser 385	Thr .	Ala '	Val '	Val :	lle 390	Trp	Pro	Arg	Ser	Leu 395	Ası	n P	ro A	usn.	Arg	Val 400
	Thr	Phe :	Lys A	Ala A	Asn <i>A</i> 105	Arg !	Pro .	Phe	Leu	Val 410	Phe	Ile	• A:	rg G		Val 415	Pro
50	Leu /	Asn 1	Chr 1	le I 20	le F	he M	det (	Gly A	Arg 425	Val	Ala	Asn	Pı		ys \ 30	√al	Lys

	(1) SEQUENCE CHARACTERISTICS.													
5	<ul><li>(1) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 464 amino acids</li><li>(B) TYPE: amino acid</li><li>(D) TOPOLOGY: linear</li></ul>													
	(ii) MOLECULE TYPE: protein													
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:													
10	Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val													
	Tyr Leu Leu Ser Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys -15 -10 -5													
15	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10													
	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30													
20	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45													
	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 . 55 60													
25	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 75 80													
	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90 95													
30	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser 100 105 110													
	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys 115 120 125													
35	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 135 140													
	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 155 160													
40	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175													
	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190													
45	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205													
	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220													
50	Trp Lys Ser Lys Phe Ser Fro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 225 230 240													
	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly													

					245					250					25	5
5	Lys	5 Phe	Arg	Туг 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gla	. Val	Leu 270		u Le
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285		Ly.	s Pr
10	Glu	1 Lys 290	Ser	Leu	Àla	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Va.	l Le
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	5 Me
15	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Let 335	
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Let	Pr
20	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Ph
20	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
05	Ser 385	Thr	Ala	Val	Val	Ile 390	Val	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
25	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	
	Leu	Asn	Thr	Ile 420	Ile	Phe	Met	Gly	Arg 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys
30	(2)	INFO	יי באם	ז רו א	FOR	550	TD N	0. 1	0.							
35	(2)		i) S (A (B)	EQUE LEI	NCE NGTH	CHAR : 46	ACTE	RIST ino id	ICS:	s						
		(ii)														
40	Met '	(xi) Tvr 5											· •			
	-32	_	- 30				-	-25				-	-20			
45		15				_	-10					-5				
	His C				5					10					15	
50	Met A			20					25					30		
	Asp G	Slu G	ly S 35	er G	lu G	ln L	ys I	le P 40	ro G	lu A	la T		sn A 45	rg A	rg '	√al

	Tr	5 G1 s		ı Ser	: Lys	: A1	a Asi		r Ar	g Ph	e Al	ā Th ō		z Ph	е Ту	r Gln
5	His 65		ı Ala	: Asp	5e:	7 (		. As	o As	n Ası	р As. 7		e Ph	e Le	u Sē	r Prc EO
	Ъer	Sei	r Ile	: Ser	Thi 85		. Phe	e Ala	e Me	t Th. 91	_	E Lei	n e3	y Al	a Cy:	s Asn 5
10	Asp	Thi	Leu	Gln 100		Leu	Met	Glu	) Val 105		E Lys	s Phe	≥ Asj	2 Th.		e Ser
	Glu	Lys	Thr 115		Asp	Glr	Ile	His 120		e Phe	Phe	: Ala	125		u Asr	ο Сλε
15	Arg	Leu 130		Arg	Lys	Ala	Asn 135		Ser	Ser	Lys	140		l Sei	r Ala	Asn
	Arg 145		Phe	G1 y	.E.sp	Lys 150		Leu	Thr	Phe	Asn 155		Thi	Туг	Glr	160
20	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Γ7.ε	Leu 170		Pro	Lei	Asp	275 175	_
	Glu	Asn	Æla	Glu 180	Gln	Ser	Arg	Ala	Ala 185		Asn	Lys	Trp	Val 190		Asn
25	Lys	Thr	Glu 195	Gly	Arç	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205		Ile	Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
30	Trp .225	Lys	Ser	-	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	GJA
35	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270	Glu	Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
40	Glu	Lys 290	Ser	L∈u	Äla	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
	Gln 305	Glu	Trp	Leu .		Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
45	Pro	Arg	Phe		11e 325	Glu	Asp	61 y	Fhe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
<del>,</del> 0	Asp	Meτ		Leu ' 340	Val.	A.s.p	Leu		Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
-0	Gly		Val . 355	žla (	Glu (	5ly .		Asp 360	Asp	Leu	Tyr		Ser 365	Asp	Ala	Ph∈
50		Lys . 370	Ala :	Phe 1	Leu (		Val 2 375	-sn	Glu	Glu		Ser 380	Glu	Ala	Ala	Ala

		5 e 3 8	r Th	r Al	a Va	l Va	1 I1 39	e Le	eu Pi	14 O	g Se	er Le 39	eu A: 95	sn Pi	ro A:	sn Ai	rg Va 40
5		Th	r Ph	e Ly	s Al	a As 40	n Ar 5	g Pi	o Pł	ne Le	eu Va 41	1 Ph	e Il	le A:	rg Gl	u Va 41	al Pro
		Le	u As	n Th	r Il 42	e Il O	e Ph	e Me	t Gl	y Ar 42	g Va !5	1 A1	a As	n Pr	0 Cy 43		l Ly:
10		(2	) IN	FORM	ATIO	N FO	R SE	Q ID	NO:	11:							
15					SEQ! (A) : (B) : (D) :	LENG: TYPE	TH: : am	464 ino	amin acid	o ac							
			(i:	i) M	OLEC	ULE 7	rype	: pr	otei	n							
					EQUE												
20		Me1 -32	ту <u>:</u> ?	- 30	r Ası O	ı Val	1 116	e Gl	y Th -2	r Vai	l Th	r Se	r Gl	y Ly.		g Ly.	s Val
		Ту	Leu -15	ı Let	u Se:	Lev	ı Let	1 Lev	ı Il	e Gly	y Phe	e Tr	As;		s Va	l Th:	r Cys
25		His 1	. ej?	/ Sei	r Pro	Val	Asp	o Ile	≘ Су	s Th	r Ala		5 Pr	o Ar	g As	9 Ile 1	e Pro
		Met	Asn	Pro	Met 20	Cys	Ile	тул	: Ar	g Se : 2:	r Pro	o Glu	ı Ly:	s Ly:	s Ala 30		Glu
30		Asp	Glu	Gly 35	/ Ser	Glu	Gln	Lys	11e	Pro	o Glu	ı Ala	Thi	Asr 45		) Arç	y Val
		Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	: Arg	Phe	: Ala	Th:		Phe	туг	Gln
35		His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75		Ph∈	: Leu	Ser	Pro 80
		Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
40	•				Gln 100					105					110		
		Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
45		Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
40		Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Туг	Gln	Asp 160
		Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
50		Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	lle	Asn	Lys	Trp	Val 190	Ser	Asn

	Lys	Thr	Gl u 195		Arg	lle	The	Asp 200		1 <u>1</u> é	Pro	, Ser	61 L 205		IÌ€	Asn
5	Glu	Leu 210		Val	Leu	Val	Leu 215		Asn	Thr	lle	Tyr 220		Ly's	G] y	Leu
	Trp 225	_	Ser	Lys	Phe	Ser 230		Glu	Asn	Thr	Arg 235	_	Glu	Leu	Phe	Tyr 240
10	Lys	λla	Asp	Gl y	Gl u 245	Ser	Cys	Ser	λla	Ser 250		Het	Tyr	Gln	Gl u 255	Сĵ'n
	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Gl u 265	Сĵу	Thr	Gln	Val	Leu 270	Glu	Leu
15	Pro	Phe	Lys 275	-	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
20	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	M≘t	Met 315	Leu	Val	Val	His	Мет 320
	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	Βĵλ	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
25	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
30	His	Lys 370	Ala	Phe.	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val		Ala 390	Val	Pro .	Arg		Leu 395	Asn	Pro	Asn	Arg	V≥1 400
35	Thr	Phe	Lys		Asn . 405	Arg	Pro	Phe		Val 410	Phe	Ile .	Arg		Val 415	Pro
	Leu	Asn	Thr	Ile 420	Ile	Phe	Met	_	Arg 425	Val.	Ala.	Asn	Pro	Cys 430	Val	Lys
40	(2)	INFO	RMAT	иои	FOR :	SEQ	ID N	D: 12	2:							
		(	(A	) LE	NCE ( NGTH PE: 4	: 46	4 am.	ino a		s						
45		(ii)	(D	) TO:	POLO	GY: .	line	ar								
		(xi)							EQ II	O NO:	: 12:	:				
50	Met -32		Ser 2 -30	Asn \	/al ]	lle (		ſhr \ ∙25	/al T	Thr S	Ser (		ys 2 20	Arg 1	Lys \	/al

	Tyr Leu Leu Ser Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys -15 -10 -5
5	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 16
	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30
10	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 60
15	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 75 80
	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90
20	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser 100 105 110
	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys 115 120 125
25	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 135 140
	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 155 160
30	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175
	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
35	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205
	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220
40	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 235 240
	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly 245 250 255
45	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270
45	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
50	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320

	Pr	o A.	g Ph	e Ar	g 11:		u As	p 6)	y Ph		er Le So	eu L	ys G	lu G	ln Le 33	au 31 5
5	As	р Ме	E Gl	y Le 34		) As	p Le	u Ph	ė Se 34		:o G]	lυ Ly	y's St	er Ly 35		u Pr
	G)	א וז	e Va. 35.		a Gl	. CJ	y Ar	g As 3ó		p Le	u Ty	r Va		er As 88	p Al	a Ph
10	Hi	ε Ly 37		a Phe	e Lei	. Gl	u Val 37:		n Gl	บ Gl	u Gl	y Se 3.6		u Al	a Al	a Ala
	Se. 38:		r Ala	e Val	l Vā]	Le:		≘ Pro	ь Ar	g S≘	r Le 39		n Pr	o As	n Ar	g Val 400
15	Th	r Phe	e Lys	s Æla	405		g Pro	Phe	e Le	u Va 41		e Il	e Ar	g Gl	บ Va 41	l Pro 5
	Ŀеı	ı Ası	Thr	: 11s		Phe	: Мет	: Gl	/ Arc 425		1 41	a As	n Pr	о Су 43		l Lys
20	(2)	INF	-07M-	TION.	FOF.	5EQ	) ID	NO:	13:							
			(	A) L E) T	ENCE ENGT: YPE:	H: 4 ami	64 a	mino ⊂id								
?5		(ii			OFOL											
		(xi	) SE	QUEN	CE D	ESCR	IPTI	ON:	SEQ	ID N	io: 1	. З :				
30		Tyr		Asn	Val	Ile	Gly	Thr -25	Val	Thr	Ser	. ej7	/ Lys		l FAs	Val
	Tyr	Leu -15		Ser	Leu	Leu	Leu -10	Ile	Gly	Phe	Trp	Asp -5	_	: Val	Thr	Cys
35	His 1	Gly	Ser	Pro	Val 5	Asp	Ile	Cys	Thr	Ala 10		Pro	Arç	Asp	Ile 15	Pro
	Met	Asn	Pro	Met 20	C7.2	Ile	Tyr	Arg	Ser 25	Pro	Glu	Lys	L;/s	Ala 30		Glu
10	Asp	Glu	Gly 35	S∈r	Glu	Gln	Lys	Il∈ 40	Èro	Glu	Ala	Thr	Asn 45		Arg	Val
	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Ph.e	Ala	Thr 60		Phe	Tyr	Gln.
15	His 65	Leu	Ala	Asp	Ser	Ly's 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
	Leu	Ser	Ile	Ser	Thr . 85	Ala	Phe	Ala	Meτ	Thr 90	Lys	Leu	GŢλ	Ala	Cys 95	Asn
0				100	Gln				105					110		
-	Glu	Lys	Thr	Ser	250 1	G l n	TIE	u: -	Dh.a	Dhe	D. L	* 3 -	7	•	_	

	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala As 130 135 140	מ
5	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln As 145 150 155 16	0
	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Ly 165 170 175	
10	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser As 180 185 190	
	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Ass 195 200 205	
15	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220	
	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 225 230 235	)
20	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly 245 250 255	
	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270	
25	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285	
	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300	
30	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320	
	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335	
35	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 . 350	
	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365	
40	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala 370 375 380	
	Ser Thr Ala Val Val Ala Tyr Pro Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400	
45	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 415	
	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430	
50		

(2) INFORMATION FOR SEQ ID NO: 14:

5				(A) : (E) :	TYPE TYPE TOPO)	ΓΗ: : am	464 i	amin acid	c. ac							
					JLE 1		•									
		( <b>)</b> : 3	.) SE	EQUE	ACE I	DESC	RJ PTI	ON:	SEQ	ID 1	10:	14:				
70	Мет - 32	_	-30		y Val	. Ile	∈ Gly	7 Th: -2!		l Th	r Sei	r Gly	- 20	_	l Lys	val
	Tyr	Leu -15		Ser	Leu	Leu	-10		≘ G]	y Phe	e Trp	Asp -5	_	: Val	The	Cys
15	His 1	_	Ser	Pro	val 5		lle	: Cys	Th	r Ala	_	e Pro	Arç	Asp	11e	Pro
	Met	A.s n	Pro	Met 20		Ile	Tyr	Arc	Se:		Glu	Lys	ГÀг	Ala 30		Glu
20	Asp	Glu	Gly 35		Glu	Gln	Lys	11∈ 40		Glu	Ala	Thr	Asn 45	-	Arg	Val
	Trp	Gl u 50	Leu	Ser	Lys	Ala	Asn 55		Arg	Phe	Ala	Thr 60	Thr	Phe	Tyr	Gln
25	His 65	Leu	Ala	Asp	Ser	Lys 70		Asp	Asn	Asp	' Asn 75		Phe	Leu	Ser	Pro 80
	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
30	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105		Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Il∈	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Суз
35	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
40	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
40	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn
	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Gl u 205	Ala	Ile	Asn
45	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Туг 220	Phe	Lγs	Gly	Leu
	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn		Arg 235	Lys	Glu	Leu		Tyr 240
50	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Туг		Glu 255	Gl y

	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270
5	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
10	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320
	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335
15	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 350
	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365
20	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala 370 375 380
	Ser Thr Ala Val Val Ala Trp Pro Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400
25	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Giu Val Pro 405 410 415
25	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430
<i>30</i>	(2) INFORMATION FOR SEQ ID NO: 15:
	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 464 amino acids  (B) TYPE: amino acid  (D) TOPOLOGY: linear
35	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:  Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val  -32 -30 -25
40	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys  -15  -10  -25  -27  Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys
	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro
45	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu
	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val
50	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 55 60

58

	His 65		Ala	Asp	. Ser	Lys 70		Asp	o Fei	Asp	> Asr 75		e Phe	: Leu	ı Ser	Pro 80
5	Leu	Ser	lle	Ser	Thr 85		Phe	⊬.la	. Met	Th: 90		Lei	e) 7	, Ala	č: Cha	Asn
	Asp	Thr	Leu	Gln 100		Leu	Met	Glv	val 105		Lys	Phe	: Asp	Thr 110		: Ser
10	Glu	Lys	Thr 115		Asp	Gln	Ile	His 120		Phe	Ph∈	Ala	Lys 125		Asn	Cys
	Arg	Leu 130		Arg	ГЛS	Ala	Asn 135		Ser	Ser	Lys	Leu 140		Ser	A] ē	Asn
15	Arg .145	Leu	Phe	GЈУ	Asp	Lys 150		Leu	Thr	Phe	Asn 155		Thr	туг	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170		Pro	Leu	Asp	Phe 175	Lys
20	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190		Asn
	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
25	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	V≥l	Asn	Thr	Ile	Туг 220	Phe	Lys	Gly	Leu
	Trp 225	Lys	Ser	Lys	Phe	5er 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
30	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
	Lys	Phe	Arg	Туг 260	Arg	Arg	Val	Æla	Glu 265	Gl 'n	Thr	Gln	Val	Leu 270	Glu	Leu
35	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
40	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
	Pro	Arg.	Phe	Arg	11e 325	Glu	Asp	Gl y	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
<i>4</i> 5	Asp	Met	Gl y	Leu 340	Val	Asp	Leu	Phe	S∈r 345	Pro		Lys · .	Ser	Lys 350	Leu	Pro
	G1 y	Ile	Val 355	Ala	Glu	Gl y	Arg	Asp 360	Asp	Leu	Tyr		S∈r 3€5	Asp	Æla	Phe
50	His	Lys 370	Ala	Phe	Leu		Val 375	As n	Glu	Glu		5er 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val	Val	Leu 390	Trp	Pro	Arg		Leu . 395	Asn	Pro.	7.5Tı	Arg	Val 400

	T	hr P	he L	ys A	la A	sn A 05	rg P	ro P	he L	eu V 4	al P 10	he I	le A	rg G		al P: 15
5	L	eu A	sn T	hr 11 41	le I. 20	le P	he M	et G	ly .≃. 4	rg V 25	al A.	la A	sn P		ys V 30	al Ly
10	(:	2) II		(A) (B)	ON FO QUENC LENG TYPE TOPO	E CH TH:	HARAC 464 nino	CTER: ami: acid	ISTI no a	CS:						
15				OLEC	ULE INCE	TYPE	: pı	rotei	in	O ID	NO:	16:				
20	_	t Ty 2	r Se -3	r As	n Va	1 11	e Gl	y Th -2	nr Va !5	al Th	ır Se	r Gl	-2	0		's Val
		-	•				- 1	U				-	5			r Cys
0.5	Hi	s Gl	y Se	r Pr	o Va	l As 5	p Il	e Cy	s Th	r Al	a Ly O	s Pr	o Ar	g A.s		e Pro 5
25	Me	t Ası	n Pr	o Mei 20	t Cy: 0	s Il	е Ту	r Ar	g Se	r Pr 5	o Gl	u Ly	s Ly	s Al 3		r Glu
	Ası	p Gli	ı Gl 3	y Ses 5	r Glu	ı Glı	n Ly	s Il 4	e Pr O	o Gl	u Ala	a Th	r Ası 45		g Ar	g Vál
30	Trp	Glu 50	ı Leı	ı Sei	Lys	Ala	Ası 55	n Se:	r Ar	g Ph	⊇ Ala	Thi 60	Thi	: Phe	≘ Ту	c Gln
	His 65	s Leu	Ala	a Asp	Ser	Lys 70	A.sr	n Asp	Ası	n Asp	Asn 75	Ile	Phe	: Lei	Ser	Pro 80
35	Leu	ser	Ile	: Ser	Thr 85	Ala	Phe	: Ala	Me t	Th:	Lys	Leu	Gly	Ala	Cys 95	Asn
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr	Ile	Ser
40	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Ąsn	Cys
	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
45	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	туг	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
50	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn

	Lys	. Thi	Glu	ı Gly	' Arg	116	- Thr	Asp	va)	11e	Fro	Ser	610	. Ala	ılle	: Asn
	•		195		,			300					205			
5	Glu	210		Val	Leu	Va)	1 Leu 215		Asn	Thr	Ile	Tyr 220		Lys	G1;	Leu
	Trp 225	-	Ser	Lys	Phe	Ser 230		G). u	Asn	Thr	Arg 235	ГÀг	Glu	Leu	Ph∈	Tyr 240
10	гÀг	Al a	Asp	ej y	Glu 245	Ser	Cys	Ser	A.l a	5er 250		Met	Ţ'nr	Gln	Glu 255	G1 y
	Lys	Phe	Arg	Туг 260	Arg	Æ.r g	Val	Ala	Gl u 265	Gly	Thr	Gln	Val	Leu 270		Leu
75	Pro	Phe	Lys 275		Asp	Asp	Ile	Thr 280	Met	Va]	Leu	Il∈	Leu 285	Pro	Lys	Pro
	Glu	Lys 290	Ser	Leu	Alā	Lys	Vāl 295	Glu	Lys	Gl u	Leu	Thr 300	Pro	Glu	Vāl	Leu
20	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	M∈t	Met 315	Leu	Val	Val	His	Мет 320
20	Pro	Arg	Phe	Arg	11∈ 325	Glu	Asp	GГÀ	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	СĵА	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	5er	Lys 350	Leu	Pro
25	Gly	Ile	Val 355	Ala	Glu	сīУ	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	_	Ser 380	Glu	Ala	Ala	Ala
30	Ser 385	Thr	Ala	Val		Ala 390	Val	Gly.	Arg		Leu . 395	A.sn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys		Asn . 405	Arg	Pro	Phe		Val 410	Phe :	Ile .	Arg		Val 415	Pro
35	Leu	Asn		Ile 420	lle	Phe	Met		Arg ' 425	Val.	Ala J	Asn :		Cys 430	Val	Lys
	121	TNFO	DM2: T	T ON T	FOD (	550	ID N	J. 1.	7.							-
40	(2)		i) S	EQUEI	NCE (	CHAR	ACTEI	RIST:	ICS:	_						
			(B	) TY	PE: a	amin	4 am: o ac: linea	i.d	3C10:	5						
45		(ii)	MOL	ECULI	E TYI	E:	prote	≐in								
		(xi)	SEQ	JENCI	E DES	SCRI.	PTI O	): SE	EQ II	) NO:	17:					
	Met '			Asn \	/al I	le		'hr \ 25	al T	hr S	er G		ys A 20	arg l	lys '	/al
50	Tyr :	Leu I -15	Leu S	Ser 1		eu !		le G	aly F	he T		sp C -5	ys V	'al 1	hr (	:ys

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	н	s Gl	y Se	r Pr	:0 Va	al A: 5	sp I	<u>l</u> e (	Cys	Thi	: Ala 10	a Lys	s Pr	o Ai	g A		le 15	Pro
5	Me	t As	n Pr	o Me	t Cy	/s 1]	le T	yr A	Arg	Ser 25	Pro	o Glu	ı Ly	s Ly		la T. 30	hr	Glu
	As	p Gl	u Gl 3	y Se 5	r Gl	u Gl	ln L	ys I	11e 40	Pro	Glu	Ala	Th		n A.	g A	rg	Val
10	Tr	p Gl	u Le O	u Se	r Ly	s Al	a A.	sn S 55	ier	Arg	Phe	: Ala	Th:	r Th	r Ph	e T	γĽ	Gln
	Ні 6	s Let 5	Al.	a As	p Se	: Ly 7	s As	sn A	sp	Asn	Asp	Asn 75	Ile	≥ Ph	e Le	u Se	er	Pro 80
15		u Sei			•	J					90					9	5	
		P Thr		100	J					105					11	0		
20	Gla	Lys	Thr 115	Sei	r Ası	p Gl	n Il	e H. 1:	is 20	Phe	Phe	Phe	Ala	Ly:		u As	n (	Cys
	Arg	130	Туг	Arç	g Lys	s Al	a As 13	n Ly 5	ys	Ser	Ser	Lys	Leu 140		Se	r Al	a ž	n a f
25						131	J					155					1	160
		Ser			100	,					170					17	5	
30		Asn		100					1	182					190	)		
		Thr	133					20	U					205				
35		Leu 210					215	)					220					
	220	Lys				230						235					2	40
40		Ala			243					2	250					255		
		Phe		200					21	65 .					270			
45			2,3					280	)					285				
		Lys : 290					295					3	00					
5 <i>0</i>		Glu 7				310					3	15					32	0
	Pro .	Arg !	Phe A	Arg :	Ile ( 325	Glu	Asp	Gly	₽h	e S	er L 30	eu L	ys G	Slu (		Leu 335	Gl	n

	Asp	Met	c Gly	y Let 340		l Asy	r Le	u Ph	e Se 34		o (3)	u Ly	s Se	r Ly 35		u Pr:
5	GJ ?	, Ile	e Val 355		∈ Glı	u Gly	y Ar	g As. 36		p Lė	u Ty	r Va	1 Se 36		p Al	e Phi
	His	Lys 370		: Phe	E Lei	ı Glı	υ Va 37.		r: 67	u Gl	u 6]	y Se 38		u Al	a Al	ā 41 a
10	Ser 385		Ala	i Vā]	Va]	1 Ala 390		e Gl	y Ar	g S≘	r Le 39		n Pr	o Asi	n Ar	9 Val
	Thr	Рhе	Lys	Ala	4 0 5	_	Pro	o Phe	: Le	ب Va. 41		e Il	e Ar	g Glı	Va:	l Pro
15	Leu	Asn	Thr	11e		: Phe	: Met	c GJ7	/ Arg 425	-	1 A)	a Ası	n Pro	Cys 430		l Lys
	(2)	INF	OPMA	TION	FOR	: SEQ	ID	NO:	18:							
20			(.	A) L B) T	ENCE ENGT YPE: OPOL	H: 4 ami	64 a	mino cid								
25		(ii			LE T											
		(xi	} SE	QUEN	CE D	ESCR	IPTI	OH:	SEQ	ID N	10: 1	8:				
	Met -32	Tyr	Ser -30	Asn	Val	Ile	Gly	Thr -25	Val	Thr	Ser	Gly	Lys -20	-	Lys	Val
30	Tyr	Leu -15	Leu	Ser	Leu	Leu	Leu -10		Gly	Phe	Trp	Asp -5	-	Vāl	Thr	C'}'s
	His 1	Gly	Ser	Pro	Val 5	Asp	Ile	Cys	Thr	Ala 10	_	Pro	Arg	Asp	Ile 15	Pro
35	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Ærg	S∈r 25	Pro	Glu	Lys	Lys	Ala 30	Thr	Glu
	Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	Il∈ 40	Pro	Glu	Ala	Thr	Asn 45	Arg	Arg	Val
40	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Ærg	Phe	Ala	Thr 60	Thr	Phe	Tyr	Gln
	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
45	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Дlа	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Sēr
50	Glu	Ly s	Thr 115	Ser	Asp	Gln	Il∈	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys

	Ar	g Le 13	и Ту О	r Ar	g Ly	s Al	a Λs 13	n L;	ys S	er S	er Ly	s Le 14	u Va O	1 3e	r Al	a Asn
5	Ar:	g Le	u Ph	e Gl	y As	р Ly 15	s Se O	r Le	eu Tl	hr Pì	ne As 15	n Gl 5	u Th	r Ty	r Gl	n Asp 160
					16.	5				17	70				17	_
10				190	,				18	35				19	0	r Asn
			193	>				20	0				20	5		e Asn
15		210	,				21:	5				220	)			y Leu
	223	,				230	,				23	5				e Tyr 240
20					243	,				25	0				25	
				260					26	5				270	)	ı Leu
25			213	)				28	0				285	•		Pro
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Gl	u Ly	s Gl	u Lei	Thr 300		Glu	ı Val	l Leu
<i>30</i>	303			Leu		310					315					320
30				Arg	325					330	)				335	
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
35			333	Ala				360					365			
		370		Phe			3/5					380				
40	Ser 385	Thr	Ala	Val	Val	Ala 390	Leu	Gly	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
					405	•	-	-		410					415	
45	Leu /	Asn	Thr	Ile 420	Il€	Ph.e	Met	Gly	Arg 425	Val	Ala	Asn		Cys 430	Val	Lys

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 464 amino acids

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						emi : YOC.										
5			-			YPE: ESCP	-			ID N	10: ]	15:				
	Met -32		Ser -30		Val	11e	G1 y	Thr		Thi	Séi	: G1;	, Lys -20	Arç	Lys	. Va
o	Туг	Leu -15		Ser	Leu	Leu	Leu -10		G17	Phe	Trp	) Asp -5		val	The	СУ
	His 1	_	Ser	Pro	Val 5	Asp	Ile	Сув	Thr	Ala 10		Pro	Arg	Asp	11e	
5	Met	Asn	Pro	Met 20		Il∈	Tyr	Arg	Ser 25		Glu	Lys	Lys	Ala 30	Thr	G1
	Asp	Glu	G1 y 35	Ser	Glu	Gln	Lys	11e 40		Glu	Æla	Thr	Asn 45		Arg	Va
0	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55		Arg	Phe	Ala	Thr 60		Ph∈	Tyz	Gl
	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75		Phe	Leu	Ser	Pr:
5	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Су:s 95	Ası
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	5 <b>€</b> :
o	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
	Arg	Leu 130	Tyr	Arg	Lys	£1a	Asn 135	Ъуs	Ser	S∉r	Lys	Leu 140	Vāl	Ser	Æla	Ası
5	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asr 160
					165	-	_		_	170					Phe 175	
0	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	<u> 191</u> a	Ala 185	I≟∈	Asn	Lys	Trp	Val 190	Ser	Asr
	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200		Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
15	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Il€	Tyr 220	Ph∈	Lys	Gly	Leu
	225	-		_		230					235				Phe	240
5 <b>0</b>	-				245					250					Glu 255	
o.	Lys	Phe		Tyr 260	Arg	Arg	Val	Ala	Gl u 265	Gly	Thr	Gln	Val	Leu 270	G) u	Leu

	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
5	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320
10	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335
	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 350
15	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365
	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala 370 375 380
20	Ser Thr Ala Val Val Gly Leu Gly Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400
	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 415
25	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430
	(2) INFORMATION FOR SEQ ID NO: 20:
30	<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 464 amino acids</li><li>(B) TYPE: amino acid</li><li>(D) TOPOLOGY: linear</li></ul>
35	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:  Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val
40	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys  -15  -10  -25  -20  Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys
	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10 15
45	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30
50	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
50	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 55 60

	ніs 65		Ala	Asp	Ser	Lys 70		Asp	e Asr	. Asp	Asn 75		Phe	Leu	Ser	P10
5	Leu	Sēr	IJ€	Ser	Thr 85		Phe	Ala	Мet	Thr 90		Leu	Gly	Ala	Cys 95	Asn
	Asp	Thr	Leu	100 61n	Gln	Leu	Mét	Glu	Val 105		Lys	Phe	Asp	Thr 110		Ser
10	Glu	Lys	Thr 115	Ser	Asp	Gln	11e	His 120		Phe	Phe	A.) a	Lys 125		Asn	Cys
	Arg	L≘u 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
15	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Туг	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	Сĵу	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	
20	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Il∈	Asn	Lys	Trp	Val 190	Ser	Asn
	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
25	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
30	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Æla	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gl'n	Val	Leu 270	Glu	Leu
35	Pro	Ph€	Lys 275	СĴĀ	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
40	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	G) u	Met	Met 315	Leu	Val	Val	His	Met 320
40	Pro	Arg	Phe	Arg	11e 325	G] u	Asp	Gly	Phe	Ser 330	L∈u	Lys	Glu	Gln	Leu 335	Gln
45	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	5er 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
45	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Æla	Phe	Leu		Val 3 <b>7</b> 5	Asn	Glu	Glu	G1 y	Ser 380	Glu	Ala	Ala	СĵА
50	Ser 385	Thr	Ala	Val		11e 390	Ala	Gl y	A.rg	Ser	Leu 395	Asn	Pro	Asn .		Val 400

	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 425
5	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430
	(2) INFORMATION FOR SEQ ID NO: 21:
10	<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 464 amino acids</li><li>(B) TYPE: amino acid</li><li>(D) TOPOLOGY: linear</li></ul>
15	(ii) MOLECULE TYPE: protein
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
	Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25 -20
20	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys -15 -10 -5
	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 10 15
25	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 25 30
	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
30	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 60
	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 75 80
35	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90 95
	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser 100 105 110
40	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys 115 120 125
	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 140
<i>4</i> 5	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 155 160
	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175
50	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
30	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205

	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	A.Sri	Thr	11e	77r		ГÀг	GЈУ	Leu
5	Trp 225	_	Ser	Lj's	Phe	Ser 230		Glu	Asn	Thr	Arg 235	Lys	G] u	Leu	Phe	Tyr 240
	Lys	Ala	Asp	61 y	Glu 245	Ser	Cys	Ser	A.la	Ser 250	Met	Met	туг	Gln	Glu 255	
10	Lys	Phe	Arg	Tyr 260	Arg	Arg	Vāl	Ala	Glu 265	G1 y	Thr	Gln	Val	Leu 270	Glu	L∈u
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
15	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
20	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
25	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Vāl	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Æla	Ala	Ala
00	Ser 385	Thr	Ala.	Val		Il∈ 390	Ala	Gly	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	L∈u	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
35	Leu	Asn	Thr	Ile 420	IJ€	Phe	Met		Arg 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys
	(2)	INFO	RMAT	NO I.	FOR	SEQ	ID N	0: 2	2:							
40		(	( )- ( E	EQUE .) LE .) TY .) TO	NGTH PE:	: 46 amin	4 am o ac	ino id		s						
45		(ii)	MOL	ECUL	Е ТУ	PE:	prot	ein								
				UENC										_		
	-32		-30					-25					-20		Lys	
		Leu -15	Leu	Ser	Leu i		Leu : -10	Ile (	Gly	Ph∈ '	Trp .	2.sp -5	Cys	Val '	Thr (	Cys

		His 1	Gly	Ser	Pro	o Va	1 A:	g a	Ile	Cvs	s Th	r L	1 = 1		D	<b>.</b>				
													10						15	
5		Met										3						30		
	1	Asp	Glu	Gly 35	Ser	Gl:	n ej	n I	ys	11e 40	Pro	o G	lu z	.1 a	The	As 4	n A 5	rg A	lrg	Val
10	7	rrp	Glu 50	Leu	Ser	Lys	s Al	a A	.sn 55	Ser	Arç	g Pł	ne A	la	Thr 60	Th	r Pl	ne I	'yr	Gln
	}	is : 65	Leu	Ala	Asp	Sei	Ly 7	s A O	sn	Asp	Asn	As	p A	sn 75	Ile	Phe	e Le	eu S	er	Pro 80
15	I	eu s	Ser	Ile	Ser	Thr 85	Al	a P	he .	Ala	Met	Th 9	r L O	ys 1	Leu	Gly	y Al		ys . 95	Asn
	A.	sp T	hr :	Leu	Gln 100	Gln	Le	u Me	et (	Glu	Val 105	Ph	e L	ys I	Phe	Asp	Th	r I.	le.	Ser
20	G	lu I	ys '	Thr 115	Ser	Asp	Glr	n IJ	le i	His 120	Phe	Ph	e P)	ne Æ	Ala	Lys 125	Le	u As	sn (	Cys
	A.	rg L 1	eu 1 30	ryr .	Arg	Lys	Ala	a As	n 1 85	L <u>v</u> s	Ser	S∈.	r L)	s I	eu 40	Val	Se	r Al	.a.	Asn
25	A: 14	rg L 15	eu F	he (	Gly	Asp	Lys 150	Se	r I	-eu	Thr	Phe	⊇ As 15	n G	lu	Thr	Ту	r Gl		.sp 160
	נו	le S	er G	lu I	Leu	Val 165	Tyr	Gl	уд	la	Lys	Let 170	ı Gl	n P	ro	Leu	Ası	> Ph 17		ys
30	G1	u As	sn A	la 0	51u .80	Gln	Ser	Ar	g A	la /	Ala 185	Ile	As	n L	ys '	Ггр	Val		rΑ	sn
	Ly	s Th	r G	lu G 95	ly A	A.rg	Ile	Th:	r A.	sp \ 00	Val	Ile	Pr	o Se	er (	51 u 205	Ala	I1	e A	sn
35	Gl	u Le 21	u T) 0	n <u>r</u> V	al I	Leu	Val	Let 215	ı Va	al A	Asn'	Thr	Ile	ту 22	r F	he.	Lys	Gl	/ Le	eu
	Tr:	p Ly 5	s Se	er L	ys F	Phe :	Ser 230	Pro	G]	lu A	sn :	Thr	Arç 235	Ly	's G	lu	Leu	Phe	Ty 24	
40	Lys	s Al	a As	p G	ly G 2	lu 5 45	Ser	Cys	Se	r A	la 5	Ser 250	Met	Мe	t T	yr (	Gln	Glu 255	Gl	· У
40	Lys	Ph e	e Ar	g T) 26	/r A 50	rg Æ	Arg	Val	Al	à G.	lu G 65	1 y	Thr	Gl:	n V	al 1	Leu 270	Glu	Le	u
	Pro	Phe	£ Lу 27	s G1 5	у А	sp A	sp	Ile	Th 28	r Me O	et V	al	Leu	Ile	e Le			Lys	Pr	0
45	Glu	Lys 290	Se.	r Le	u Al	la L	ys Y	Val 295	Gli	u Ly	s G	lu	Leu	Th:	Pr	0 G	lu	Val	Lei	u
	Gln 305	Glu	Tr	Le	u As	5p G 3	lu 1 10	Leu	Glı	ı G1	u Me	et 1	Met 315	Leu	v Va	1 V	al	His	Met 320	
50	Pro	Arg	Phe	e Arq	g Il 32	e G 5	lu A	Asp	G1 y	' Ph	e Se 33	er I	Leu	Lуs	Gl	u G		Leu 335	Glr	1

	À:	sp Me	et G		€⊔ \/ 40	el A	sp L	æu F		Ser :	Fro	Glu	Lyיኗ	Ser	Lys 350		u Pr
5	G)	ly II	le Va 35	al A 55	la G	lu G	1 y A		.sp /	sp 1	Leu :	ryr '		Ser 365	Asp	A.I	a Ph
	Hi	is Ly 37		la Pl	he L	≘ນ G		al A 75	sn G	Slu (	Slu (		Ser 380	G] u	Al e	<i>F</i> .1	a Al
10	S e	r <b>T</b> h	ır Al	la Pi	ne Va		le A 90	la G	ly A	rg S		eu 2 95	4sn	Pro	Asrı	٨r	9 Va 40
	Th	r Ph	e Ly	's Al	la As 40	n Ai )5	g P.	ro P	he L		'al F 10	he I	[]e /	4.rg	Glu	Va:	
15	Le	u As	n Th	r 13 42	e Il 20	e Ph	ie Me	et G		rg V 25	al A	la 4	esn I		Cys 430	Val	l Ly:
	(2	) IN:	FORM	ATIC	N FO	R SE	Q II	NO:	23	:							
20				(A) (B)	UENC LENG TYPE TOPO	TH: : am	464 ino	amin acid	o ad								
					ULE		•										
25	Met -32	туг		r As	NCE : n Va:				r Va					ys .≇ 20	rg	Ly <sub>.</sub> s	Val
30			. Lei		r Lev	je≀	Lei 11-	u Il		y Ph	∈ Tr				al '	Thr	Cys
-	His 1	e5?.	Ser	: Pro	Val		o Ile	e Cy	s Th	: Al 1		s Pr	o Ar	rg A	.sp :	Ile 15	Pro
35	Met	Asn	Pro	Me't 20	Cys	Ile	ту:	r Arq	g Se		o Gl	u Ly	's L		la : 30	Thr	Gl u
	Asp	Glu	Gly 35	Ser	· 61 u	Gln	Lys	3 11e		o Gl	u Al	a Th		n A.	rg A	Arg	Val
10	Trp	Gl u 50	Leu	Ser	Lys	Ala	Asn 55	Ser	: Arq	g Phe	≥ Al:	∓ Th 6		r Pl	he I	,Àī	Gln
	His 65	Гел	Ala	Asp	Ser	Lys 70	Asn				7 . 7		e Ph	€ Le	eu S	er	Pro 80
	Leu	Ser	Ile	Ser	Thr 85		Phe	Ala	Met	Th:		Lei	u Gl	y Al	la C	y'≅ . 95	Asn
15	Asp	Thr	Leu	Gl n 100	Gln	Leu	Met	Glu	Val 105		Lys	Phe	≗ Asj	p Th		le :	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	125		u A	sn (	Cys
50	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140		l Se	r A	la .º	Asn.

	Ar 14	g Lei 5	ı Phe	e Gly	y Ası	2 Ly 15	s Se O	r Le	u Th	r Phe	As:		u Th	г Ту	r Gl	n Asp 160
5	110	e Se	r Gli	ı Let	va) 165	l ту	r Gl	y Al	a Ly	s Let 170	ı Glı )	n Pr	o Le	u As	p Phe 17	e Lys
	Gli	u Asr	n Ala	180	ı Glr	se.	r Ar	g Al	a Al 18	a Ile 5	e Ası	ı Ly:	s Tr	9 Va.		r Asn
10	Lys	5 Thi	Glu 195	g Gly	' Arg	; I1	· ∋ Th.	20	p Va O	l Ile	Pro	Se:	r Gl v 205	ı Ala	a Ile	Asn
	Glı	210	Thr	: Val	Leu	Va]	1 Let 21:	u Va.	l As:	n Thr	Ile	220		E Lys	s Gl)	/ Leu
15	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Gl:	ı Ası	n Thr	Arg 235	Lys	s Glu	Let	) Phe	Tyr 240
75	Lys	Ala	Asp	Gly	Glu 245	Ser	Cy's	Se:	Ala	s Ser 250	Met	Met	Туг	Glr	Glu 255	
	Lys	Phe	Arg	Туг 260	Arg	Arg	(Val	Ala	Glu 265	Gly	Thr	Gln	ı Val	Leu 270		Leu
20	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Th: 280	Met	: Val	Leu	Ile	Leu 285		Lys	Pro
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Gl u	Lys	Glu	Leu	Thr 300		Glu	Val	Leu
25	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met.	Met 315	Leu	Val	Va l	His	Met 320
	Pro	Arg	Phe	Arg	11∈ 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
30				340					345	Pro				350		
			355					360		Leu			365			
35		370					375			Glu		380				
	365					390					395					400
40	Thr	Phe	Lys	Ala .	Asn . 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
	Leu .	Asn	Thr	Ile : 420	Ile	Phe	Met	Gly	Arg 425	Val .	Ala	Asn		Cys 430	Vaļ	Lys
45	(2)	INFO	RMAT:	ION E	FOR S	SEQ	ID N	O: 2	4:							
			L) SI	EQUEN	ICE (	CHAR	ACTE	RIST	ICS:	s						
5 <i>0</i>			(B)	TYF	E: a	min	o ac	id								

		(i:	i) M	DEC	ULE	TYPE	: pr	otei	n							
		(x:	i) SI	EQUE	VCE	DESC	RIPT	1 ON :	SEC	ID	но:	24:				
5	Me t - 33		- Se:		ı Va.	1 11	e G1;	y Th -2		1 Th	r Se	r Gl	y Ly -2		g Ly	s Vā]
	Туг	Let -15		Se:	Lei	Le ا	u Lei -l(		e Gl	y Ph	e Tr	p As		s Vā	1 Th	r Cys
10	His 1		, Ser	Pro	Val		o Ile	e Cy	s T'n	r Al l		s Pr	o Ar	g As	p Il 1	e Pro 5
	Met	Asn	Pro	Met 20	-	: Ile	e Ty1	Ar	g Se 2		o Glı	Ly:	в Гу.	s Ala 3		r Glu
15	Asp	Glu	Gly 35		Glu	Glr	n Lys	11 4		o Gl	u Ala	Thi	4.		g Ar	g Val
	Trp	Glu 50		Ser	Lys	Ala	Asn 55		r Ar	g Ph	e Ala	Thr 60		r Phe	ty:	Gln
20 .	His 65	Leu	Ala	Asp	Ser	Lys 70		Asp	o Asr	n Asp	Asn 75		Phe	e Leu	ı Seı	Pro 80
	Leu	Ser	Ile	Ser	Thr 85		Phe	Ala	мет	Th:		Leu	Gly	Ala	Cys 95	Asn
25	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105		: Lys	Phe	Asp	Thr 110		ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120		Phe	Phe	Ala	Lys 125		Asn	Cys
30	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	ej A	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
35	Ile	Ser	Glu	Leu	Val 165	Туг	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
	Glu	Asn	Ala	Gl ប 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn
40	Lys	Thr	Glu 195	ej λ	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
		Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Il€	Туг 220	Phe	Lys	ĠΙλ	Leu
45	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
	Lys .	Ala.	A.sp		Gl u 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	
50	Ly's ·	Phe.		Tyr . 260	Arg	Arg	Val.	Ala	Glu 265	Gly	Thr	Gln	Vāl	Leu 270	Glu	Leu

	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pr
5	Glu	1 Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Va]	Le
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Me <sup>-</sup> 32
10		Arg			325					330					335	
		Met		340					345					350		
15		Ile	333					360					365			
		Lys 370					3/5					380				
20	_ 303	Thr				390					395		g*	_		400
		Phe			405					410					415	
25	Leu	Asn	Thr	11e 420	Ile	Phe	Met	Gly	Arg 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys
	(2)	INFO	RMAT	ION	FOR	SEQ	ID N	0; 2	5:							
30		(	(A (B	EQUE ) LE: ) TY ) TO:	NGTH PE:	: 46 amin	4 am o ac		ICS: acid	s						
		(ii)														
35	Met :	(xi) Tyr S					aly 1					Ely 1	Lys A	arg 1	Lys '	Val
10	Tyr 1	Leu I -15	eu S	Ser I	eu I	eu I	eu 1	le G	51 y F	he I	rp A			al T	Thr (	Cys
40	His C	Gly S	er P	ro V	'al A 5	sp I	le C	ys T	hr A	la L 10	ys P	ro A	Arg A	sp I	le 1	Pro
· .·	Met A	Asn P	ro M	et C 20	ys I	le T	yr A	rg S	er P 25	ro G	Ĭu L	ys I		la T 30	hr C	lu
45	Asp G	Slu G	ly s 35	er G	lu G	ln L	ys I	le P 40	ro G	lu A	la T	hr A	sn A 45	rg A	rg V	al
	Trp G	lu L	eu S	er L	ys A.	la A	sn S 55	er A	rg Pl	he A		nr T 50	hr Pl	ne T	yr G	ln
50	His L 65	eu A	la As	sp Se	er L	ys A: 70	sn A:	sp As	sn As	sp As	sn Il 75	le Pi	he Le	èu Se		ro 80

	Lev	ı Sei	r Ile	: Sei	Th:		a Fhe	≥ A1.	a Me	t Th		s Le	u Gl	y A1	a Cy:	a Asn
5	Asp	tdT (	Leu	100		Lēi	ı Met	: Gl	u Vā. 10		≥ Ly:	s Phe	e As	p Th 11		e Ser
	Glu	Ly:	Thr 115		Asp	Glr	ıle	Hi:		€ Ph∈	e Pin	÷ .A1 a	12:		u Asr	cys c
10	Arg	leu 130	-	Arg	ьуs	Ala	Asn 135	-	s Se:	r Sei	: Lys	140		l Se:	r Ala	Asn
	Arg 145		Phe	Gly	Asp	Lys 150		Leu	Th:	: Phe	: Asr 155		Th:	ту:	r Glr	160
15	Ile	Ser	Glu	Leu	Val 165	_	Gly	Ala	Lys	170		Pro	Lev	ı Asp	Phe 175	-
	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185		Asn	Lys	Trp	Va)		Asn
20	Ъуs	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200		lle	Pro	Ser	G1 u 205		Ile	Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
25	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
	Lys	Ala	Asp	Gly	Glu 245	Ser	Сув	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
30	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270	Glu	Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
35	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295		Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Геп
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
40	Pro	Arg	Phe	Arg	Il∈ 325	Glu	Asp	Gly	Phe	S∈r 330	Leu	Lys	Glu	Gln	Leu 335	Gln
40	Asp	Met			Vāl.						Glu	-		Lys 350	Leu	Pro
	Gly	Ile	Val . 355	Ala	Glu	Gl y		Asp 360	Asp	Leu	Туг	Val	Ser 365	Asp	Ala	Phe
45		Lys 370	Ala	Phe	Leu		Val . 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala '	Val		Ile . 390	A.la	G] Y	Arg		Leu . 355	Asn.	Arg	Arg	Arg	Val 400
50	Thr	Phe	Lys :		Asn ) 405	Arg :	Pro :	Phe		Val 410	Ph∈	Ile .	Arg		Val 415	Pro

Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430

5	12	1 TN	FORM	חזית מ	N FO	R SE	O 7.0	NO.	26.							
10	``_	, 1	(i)	SEQ (A) (B)	UENÇ LENG TYPE	E CH TH: : am LOGY	ARAC 464 ino	TERI amin acid	STIC o ac	S:						
		(i	i) M	OLEC	ULE '	TYPE	: pr	otei	n							
		(×.	i) S	EQUEI	NCE :	DESC	RIPT	ION:	SEQ	ID 1	10:	26:				
15	Me: -3:	t Ту. 2	r Se:	r Ası O	n Va.	1 11	e Gl	y Thi	r Va	l Th	r Se:	r Gl	y Ly: -2(		g Ly:	s Val
	Ту	r Lei	u Lei 5	ı Se	r Lei	u Lei	1 Let	ı Ile	e Gl	y Phe	e Trp	Asp -!		val	Th	r Cys
20	His	G G 1 <u>1</u>	y Sei	Pro	Va.	l Asp	o Ile	e Cys	Th	r Ala		s Pro	Arg	Asp	116	Pro
	Met	Asr	Pro	Met 20	Cys	s Ile	Э Туг	r Arg	Se:		Gli	ı Lys	s Lys	Ala 30		Glu
25	Asp	Glu	35	; Ser	Glu	ı Glr	n Lys	40	e Pro	o Glu	Ala	Thi	: Asr 45		Arç	y Val
	Trp	61 to 50	Leu	Ser	Lys	: Ala	Asn 55	Ser	Arg	, Phe	Ala	Th.:		Phe	Туг	Gln
30	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75		Phe	Leu	Ser	Pro 80
					85					90					95	
35	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125	Leu	Asn	Cys
40	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe ï	Asn 155		Thr	Tyr	Gln	Asp 160
45	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
				180		Ser			185					190		
50	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn

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ANTER THE STEE OFFICE STATES

	Gl	u Let 210		: Val	Léu	ı Val	1 Leu 215	Val	. Asn	Th:	: Ile	Ty:		e Ly	s Gl	y Leu
5	Tr:		: Sei	Lys	Phe	Se1 230		6lu	A.S.n	Tr: r	Arg 235		Gli	u Le	ս Բե	e Tyr 240
	Lys	s Ala	Asp	Gly	Glu 245		Cys	Ser	Ala	Ser 250		Met	Ty	r G1:	61: 25:	o Gly
10	Lys	: Phe	Arg	Tyr 260		Arg	Val	Ala	G1 u 265		Thr	Gln	Va]	Let 270		ı Leu
	Pro	Phe	L;'s 275		Asp	Asp	Ile	Thr 280		Val	Leu	Ile	Leu 285		ь Гуз	Pro
15	Glu	Lys 290		Leu	Ala	Lys	Val 295	Glu	Lλε	Glu	Leu	Thr 300	Pro	Gl u	(Va)	Leu
	Gln 305		Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Vāl	۷al	His	320
20	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	C] À	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Ly's	Ser	Lys 350	Leu	Pro
25	Gly	Ile	Val 355	Ala	Glu	GJ À	Arg	Asp 360	Asp	Leu	Tyr	Val	5er 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu		S∈r 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val		Ile 390	Ala	Pro	Arg	Ser	Leu . 395	Asn	Pro	Asn	Arg	Val 400
30	Thr	Phe	Lys		Asn . 405	Arg	Pro	Phe		Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
	Leu	Asn		Ile 420	Ile	Phe	Met		Arg ' 425	Val.	Ala :	Asn	Pro	Cys 430	Val	Lys
35	(2)	INFO	TAMS	I ON	FOR :	SEO	ID N	D: 2'	7:							
40			i) S (A (B	EQUEI ) LEI ) TYI	NCE ( NGTH: FE: a	CHAR : 46	ACTE 4 am: o ac: linea	RIST: ino a	ICS:	3						
						_	orote								•	
45	Met	Tyr :	Ser 2				PTION Sly T	hr V	_				ن ≥′ز۔	Arg .	Lys	Val
		Leu I	-30 Leu S	Ser 1	Jeu l		∍eu I	25 l∈ G	ly P	he T		sp C	20 'ys \	/al :	Thr	Cys
50	His (	-15 Gly :	Ser H	°ro /	'al A 5		le C	ys T		la <u>L</u> 10		-5 ro A	rg Æ	sp ]	12e 15	Pro

	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala 20 25 30	ı Thr Glu
5	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg 35 40 45	Arg Val
	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe 50 55 60	Tyr Gln
10	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu 65 70 75	0.8
	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala 85 90	95
15	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr 100 105 110	
	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Ala Gln Leu 115 120 125	
20	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser 130 140	
	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr 145 150 155	160
25		175
	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val : 180 185 190	
30	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala 1 195 200 205	
	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys G 210 215 220	
35	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu P 225 230 235	240
		55
40	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu G. 260 265 270  Pro Phe Lys Gly Asp Arg Ll The Company	
	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Ly 275 280 285  Glu Lys Ser Leu Na Lys Val Gly	
45	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Va 290 295 300	
	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val Hi 305 310 315  Pro Arg Phe Arg Ile Glu Asp Clu Des Glu Des Gl	320
50	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Le 325 330 33	5
	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Let 340 345 350	u Pro

55

 $P^{10}D + 5 + 5D + 4D + 4D = -100 (Ref + 4\Delta A - 4 - 4)$ 

	G1;	y Il	e Va 35		a Gl	u Gl	y Ar	g Asj 36		p Le	u Tj	r Va	1 Se 36		p Al	a Ph
5	Hi:	s Lys 370		a Ph	e Le	u Gl	u Va 37		n Gl	υ Gl	u Gl	у Se 38		u Al	a Al	a Al
	Se:		r #1:	ā Vā	) Va.	1 Il: 39		e Pro	s Ar	g Se	r Le 39		n Pi	o As	n Ar	g Va.
10	Thi	r Phe	≞ Ly:	s Ala	a Asi 40:		g Pro	o Phe	e Le	и Va 41		e Il	e Ar	g Gl	u Val 41	-
	Lev	) Asr	Thi	11e 420		e Phe	e Mei	c Gly	42.		l Al	a As	n Pr	5 Cy:	s Val	l Lys
15	(2)	INF			N FOF											
20			(	A) I B) T	JENCE LENGT LYPE : LOPOL	TH: 4 ami	.no a	mino cid								
					LE T		-			ID N	10: 2	28:				
25	Met -32	_	Ser -30		Val	Ile	Gly	Thr -25	Val	Thr	Ser	Gl?	/ Lys		l Lys	Val
	Tyr	Leu -15	Leu	Ser	Leu	Leu	Leu -10		Gly	Phe	Trp	Asp -5	_	Val	Thr	Cys
3 <b>0</b>	His 1	Gly	Ser	Pro	Val 5	_	Ile	Cys	Thr	Ala 10	-	Pro	Arg	Asp	Ile 15	Pro
	Met	Asn	Pro	Met 20	_	Ile	Tyr	Arg	Ser 25		Glu	Lys	Lys	Ala 30	Thr	Glu
35	Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	Ile 40	Pro	Glu	Ala	Thr	Asn 45	Arg	Arg	Val
40	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	A.1 a	Thr 60		Phe	Tyr	Gln
40	65			•		70				•	75				Ser	8 0
45					85					90	-		-		Cy's 95	
		•		100					105					110	lle	
50 .			115					120					125		Asn	
	AIG	130	ıyr	Arg	тЛг		135	гàг	>er	ser		140	val	ser	Ala	ASD

		g Lei 5									100	'				1€0
5		e Ser								170					175	
		u Asn							103					190		
10	Lys	5 Thr	Glu 195	G1 y	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
		Leu 210					213					220				
15	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
		Ala								250					255	
20		Phe							265		•	1.		270		
								200					285			
25		Lys 290				,						300				
		Glu			•	310					315				;	320
30		Arg							٤	330				3	35	
	Asp		_					-	343				3	50		
35	Gly		_				•	,00				3	65			
							, 5				3	80				
40	Ser 7 385									33	75				4	00
	Thr P				_				4 1	LU				41	.5	
45	Leu A	sn Tl	11 11 42	.e I <u>l</u> ?0	e Ph	ie Me	et G	ly A: 41	rg <sup>°</sup> Va 25	:l Al	a As	n Pr	O Cy 43		l Ly	's
	(2) II	NFORM	OITA	N FO	R SE	Q ID	NO:	29:								
50			(A) (B)	UENC! LENG' TYPE : TOPO!	rh: -	464 ino .	amin acid	o ac	S: ids							

80

			-,				· P-	0 - 0	•							
		{ ×	i) S	EQUEI	NCE	DESC	RI PT	: 40 E	SEQ	2 D	HO:	29:				
5	Ме: -33		r Se.		n Val	1 11	e Gl	y Thi -25		l Th	r Se	r G1	у Ъу -2		ġ Ly	s Va
	Ty	-1:		ı Se:	Lei	u Let	J Let -10		e Gly	y Ph	e Tr	•	բ Cy 5	s Vā	l Th	r C)
10	His 1		y Sei	Pro		Asp 5.	o Ile	÷ С7.≊	Th:	: Al:	_	s Fr	o Ar	g As	p Ile 1	
	Met	: Ası	n Pro	) Met 20		: Ile	∌ Туг	Arg	Set 25		o Gli	u Ly	s Ly:	5 Ala 30		r Gl
15	Asp	Glu	ر G1 35		Glu	Glr	Lys	11e 40		Gl:	ı Ala	a Th.	r Ası 45		g Aro	y Va.
	Trp	Gl: 50		Ser	Lys	Æla	Asn 55		Arg	Phe	Ala	3 Th:		Fhe	E Tyı	Gl:
20	His 65		Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	75		Phe	: Lei	ser	Pr:
	Ъeu	Ser	Ile	Ser	Thr 85		Phe	Ala	Met	Thr 90		Let	el?	Ala	Cys 95	
25	Asp	Thr	Leu	Gln 100	Glr	Leu	Met	Glu	Val 105	Phe	Lys	Phe	: Asp	Thr 110		Sei
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125		Asn	Суз
80	Arg	Leu 130		Arg	Lys	Æla	Asn 135	Lys	Ser	Ser	Lys	Leu 140		Ser	Ala	Asn
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Gìu	Thr	Tyr	Gln	Asp 160
35	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn
	Lys	Thr	Glu 195	el ?.	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	.Ile	Asn
o .	Glu	Leu 210	Thr	Vāl	Leu	Val	Leu 215	Val .	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
	Trp 225	Ly's	Ser	Lys	Phe	S∈r 230	Pro	Glu .	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Туг 240
15	Lys	Ala	Asp		Glu 245	Ser	cye	Ser.		Ser 250	Met	Met	Tyr	Gln	Gl u 255	Gly
•	Lys	Phe		Tyr . 260	Arg .	Arg	Val.		Glu ( 265	GJ Y	Thr	Gln	Val	Leu 270	Glu	Leu
50	Pro		Lys 275	Gly .	Asp.	Asp	lle '	Thr ! 280	√et '	Val	Leu		Leu 285	Pro	Lys	Pro

		lu Ly 29					2,5	,				300				
5		ln Gl 05				510					315					320
	P	ro Ar	g Phe	Arg	I1∈ 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	
10	A	sp Me	= Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	G.	ly Ile	≥ Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
15	нз	is Lys 370	Ala )	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
	S∈ 38	er Thr	Ala	Val	√al .	Ala 390	Leu	Gly	Arg	Ser	Leu . 395	Asn	Pro	Asn	Arg	Val 400
20	Th	r Phe	Lys	Ala A	Asn . 105	Arg	Pro	Phe	Leu	Val 410	Phe	Ile .	Arg		Val 415	Pro
	Le	u Asn	Thr	Ile 1 420	le :	Phe	Met	Gly	Arg 425	Val 2	Ala A	Asn		Cys 430	Val	Lys
25	(2	) INF	ORMAT	ION F	OR S	SEQ	ID N	0: 3	0:							
30			(B)	EQUEN LEN TYP TOP	GTH: E: a	46 mino	4 am.	ino a id	ICS: ecids	5						
			MOLE													
35	Met -32	Tyr	SEQU Ser A				ly T					ly L	ys A 20	rg L	ys \	/al
	Tyr	Leu -15	Leu S	er Le	eu Le	eu L -	eu I 10	le G	ly P	he T	rp As	sp C	ys V	al T	hr C	ys
40	His 1	Gly	Ser P	ro Vā	1 As	sp I	le C	ys Tl	hr Al				rg A			ro
	Met	Asn i	Pro M	et Cy 20	s <u>11</u>	.е т	уг А.	rg Se	er Pr 25				ys Al			lu
45	Asp	Glu (	31y Se 35	er Gl	u Gl	n Ly	ys II	le Pr 10	co G1	u Al	a Th	r As	n Ar	g Ar	g V	al
	Trp	Glu I 50	eu Se	r Ly:	s Al	a As	sn Se	er Ar	g Ph	e Al	a Th 6	r Th O	r Ph	е Ту	r Gl	Ln.
50	His 65	Leu A	la As	p Sei	Ly:	s As O	n As	p As	n As	p As:	n Ile 5	e Ph	e Le	u Se		0

	Leu	Ser	IJ÷	Sêr	Thr 65	Дlа	Phe	Als	Met	Thr 90		Leu	Gly	Ala	Cys 95	Asn
	Asp	Thr	Leu	300 300	Glrı	Leu	Met	G) u	Va) 105	Ph∈	Lys	Phe	Asp	Thr 110	116	Ser
5	Glu	Lys	Thr 115	Ser	Asp	Gln	11€	His 120		P'n∈	Phe	Ala	Lys 125	Leu	Asn	Cys
	Arg	Leu 130		Gln	Asn	Alā	Asn 135	Lys	Ser	Ser	Lys	Ъеи 140	Vāl	Ser	Ala	Asn
10	Arg 145	Leu	Phe	G) y	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	Сĵу	Alā	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
15	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Æla 185	Ile	Asn	Г7,:s	Trp	Val 190	Ser	Asn
	Lys	Thr	Glu 195	Gly	Arg	lle	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Άla	Ile	Asn
20	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	lle	Tyr 220	Phe	Lys	GJλ	Leu
·	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
25	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270	Glu	Leu
30	Pro	Phe	Lys 275	G1 y	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro <sub>.</sub>	Lys	Pro
	Glu	Lys 290	Ser	L∈u	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
35	Gln 305	Glu	Trp	L∈u	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
	Pro	Arg	Phe	Arg	11∈ 325	Glu	Asp	ely	Ph∈	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
40	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys.	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Туг	Val	Ser 365	Asp	Ala	Phe
45	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val	Val	11∈ 350	Ala	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
50	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro

	1	Leu I	Asn 7	Chr ]	11e :	lle :	Phe .	Met	Gly	Arg \ 425	Val A	Ala A	Asn !	Pro (	ys \	/al	Lys
5	(	(2) ]	NFOF	ITAN	ON E	FOR S	SEQ :	ID N	0: 3	1:							
10		ſ		(A) (B) (D)	TYP TOP	ICE OF STATES OF	464 mino Y: 1	am: ac: inea	ino a id er	ICS: acids	:						
										Q ID	NO:	31:					
15	M-		yr S					1у т		al T			ly Ly	ys A: 20	rg L	ys ì	/al
	Т	yr L -	eu Le 15	eu Se	er L	eu L	eu L -	eu I 10	le G	ly P	ne T	rp As	5p C)	rs Va	al Ti	ır (	:ys
20	Hi	s G:	Ly Se	er Pi	:0 Va	al As 5	5p I.	le C	ys T	hr Al	a Ly	/s Pr	:0 Ar	g As		.e F	,ro
	Me	t As	n Pr	0 Me	t Cy	/s Il	e Ty	yr A	rg Se	er Pr 25	o Gl	u Ly	s Ly		a Th	r G	;lu
25	As	p Gl	u G1 3	y Se 5	r Gl	u Gl	n Ly	ys I	le Pr	ro Gl	u Al	a Th	r As 1	n Ar 5	g Ar	g V	'al
							-	J		g Ph		6	0				
30						,	U			n As	/	5				ŧ	80
						_				t Th:	J				99		
35									10					110	)		
								12	U	e Phe			125				
40							155	,		: Ser		140					
						100				Phe	152				-	16	0
45					103					Leu 170					175		
									163					190			
50								200		Ile			205				
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Va]	A.s n	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu	

	Trp 225		5er	Ьуs	Phé	Ser 230		Glu	Asn	The	Arg 235		Glu	Leu	∍તેલ ા	Tyr 240
5	Lys	: Ala	Asp	GJ y	G) u 245	Ser	Cys	Ser	Ala	Ser 250		Met	Tyr	61:	Glu 255	Gly
	Lys	Phe	Arg	Туг 260	Arg	Arg	Vē]	Ala	Gl u 265	_	Thr	Gln	Val	Leu 270		L∈u
10	Pro	Phe	Lys 275	G] y	Asp	Asp	Ile	Thr 280	Met	Val	Leu	lie	Leu 285		Lys	Pro
	Glu	Lys 290	Ser	Leu	ÆĴa	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Vāl	Leu
15	Gln 305	Gl u	Trp	Leu	Asp	Glu 310	Leu	G] ŋ	Glu	Met	M∈τ 315	Leu	Val	Val	His	Met 320
	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	GJ Å	Phe	Ser 330	Leu	Lуs	Glu	Gln	Leu 335	Gln
20	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
25	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	GЈŻ	Ser 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val	Val	Ile 3 <sub>,</sub> 90	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
30	Thr	Ph∈	Lys	Ala	Asn 405	Arg	Pro	Phe		Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
	Leu	Asn	Thr	Ile 420	Ile	Phe	Met		Arg 425	Val .	Ala.	Asn	Pro	Cys 430	Val	Lys
35	(2)	INFC	ጥደሥር	TON	FOR	570	וו חד	ი. ვ	9.						•	
	(2)						ACTE									
40		·	(A (B	) LE	NGTH PE:	: 46 amin	4 am o ac line	ino id		s						
		(ii)	MOL	ECUL	E TY	PE: 1	prot	ein								
		(xi)	SEQ	UENC.	E DE	SCRI	PTIO	4: S	EQ I	D NO	: 32:	:				
45	Met -32		Ser .	Asn '	Val 1	Ile (		Thr \ -25	Jal :	Thr S	Ser (		Lys 2 -20	rg :	Lys '	Val
		Leu -15	Leu .	Ser 1	Leu I		Leu 1 -10	ile (	5ly I	Ph∈ T	îrp A	Ssp (	Cys \	Jal :	Thr (	Cys
50	His l	Gly	Ser 1	Pro \	/al <i>A</i> 5	sp l	le C	lys T	hr A	41a 1 10	ys F	ro A	rg A	asp 1	(le 1 15	Pro

	Me	et As	in Fi	o Me 2	t Су: 0	s Il	∈ Ту	r A.	rg S	er F 25	ro (	5lu	Lys	Ly		a T':	ır Glu
5	Д.	sp Gl	u Gl	y Se 5	r Glu	ı Gl	n Ly	s I	le P 10	ro G	lu A	:la	Thr	As:		g Ar	g Val
	Tr	p G1	u Le	u Se	r Lys	s Al	a As 5	n Sé 5	≥r A	rg P	he A	la '	Thr 60	Th.	r Ph	е Ту	r Gln
10	Hi 6	s Le 5	u Al	a Ası	p Ser	Ly.	s As O	n As	p A	sn A	sp A	.sn :	Ile	₽h€	e Le	u Se	r Pro 80
	Le	u Se	r Il	e Sei	Th: 85	Ala	a Ph	e Al	a Me	et T	hr L 90	ys 1	сеп	eJ 7	/ Al	а Су 9	s Asn 5
15	As	p Th	r Le	100	Gln	Let	ı Me	t Gl	u Va	11 P)	ne L	ys E	'he	Asp	Th.		e Ser
	G1	u Ly	s Th.	r Ser	Asp	Glr	ı Ile	∍ Hi 12	s Ph O	e P	ne P	he A	la	Lys 125		Ası	n Cys
20	Ar	g Lei 130	и Ту: )	Gln	Asn	Ala	Asr 135	Ly	s Se	r Se	r L		eu 40	Val	Sei	: Ala	a Asn
		•				150	_				1:	55					1 Asp 160
25					Val 165					17	0					175	<b>.</b>
				100	Gln				18	5					190		
30			195		Arg			200	1					205			
		210			Leu		215					22	20				
35	223					230					23	5					240
					Glu 245					250	)					255	_
40				200	Arg /				265						270		
			213		Asp A			280					2	85	-		
45		290			Ala I		295					30	0				
	505					310					315						320
50				-	lle G 325					.330						335	
	Asp :	Met	Gly	Leu \ 340	/al A	sp I	Leu !	Phe	Ser 345	Pro	Glu	Lys	Se		ys 1 50	Leu :	Pro

	GJУ	Ile	Val 355		G] u	Gly	Arg	Asp 360		Leu	Tyr	Val	3 6 5	⊁sp	Ala	Phe
5	His	Lys 370		Phe	Let	Glu	Val 375		61 u	Glu	e1 2.	5er 380	Glu	Ala	Ala	A.l a
	Ser 385	Thr	Ala	Vāl	Va)	390	Val	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
10	Thr	Phe	Lys	Ala	Asn 405		Pro	Phe	Leu	Val 410		Ile	Arg	G] v	Val 415	Pro
	Leu	Asn	Thr	11e 420	lle	Phe	Met	Gly	Arg 425	Va)	Аlа	Asn	Pro	Cys 430	Vāl	Lyrs
15	(2)						ID I									
			(.	A) Li B) T	engti Ype:	H: 4 ami:	RACT! 64 ar no ac line	mino cid								
20			-				prot PTIC		SEQ 1	ID N	D: 30	3:				
25	Met -32		Ser -30	Asn	Val	Ile	Сĵλ	Thr -25	Val	Thr	Ser	Gly	Lys -20	Arg	ГÃЗ	Val
	туг	Leu -15	Leu	Ser	Leu	Leu	Leu -10	Ile	Gly	Phe	Trp	Asp -5	Cys	Val	Thr	Cys
30	His 1	Gly	Ser	Pro	Val 5	Asp	Ile	Суs	Thr	Ala 10	Lys	Pro	Arg	Asp	Ile 15	Pro
	Met	Asn	Pro	M∈t 20	C)'s	Ile	Tyr	Arg	Ser 25	Pro	Glu	Ly's	Lys	Ala 30	Thr	Glu
<b>3</b> 5			35				Lys	40					45			
	_	50					Asn 55					60				
40 .	65					70	Asn				75					80
					85		Phe			90			•		95	
<i>4</i> 5				100			Met		105					110		
			115				Ile	120					125			
50		130					Asn 135					140				
	Arg 145	Leu	Phe	Gľλ	Asp	Lуs 150	Ser	Leu	Thr	₽ħ∈	Asn 155	Glu	Thr	Тут	GID	160

- 55

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5									10.	,				19	0	r Asn
								200	,				20	5		e Asn
10								•				220	J			y Leu
											235	•				e Tyr 240
15										230					25	
									200					270		l Leu
20	Pr	o Phe	275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
	Gli	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
25	Glr 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
30	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Ph∈	Ser : 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp . 360	Asp 1	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
35	His	Lys 370	Ala	Phe	Leu (	Glu	Val . 375	Asn (	Glu G	Slu (	Gly :	Ser 380	Glu	Ala.	Ala	Ala
	Ser 385	Thr	Ala	Val '	Val A	la :	Leu (	∃ly A	Arg S	er 1	Leu A 395	Asn .	Pro.	Asn .		Val 400
ro					Asn A 105				4	10				4	115	
	Leu	Asn '	Thr :	Ile I 420	le P	he N	1et G	ly A 4	rg V 25	al A	la A	sn F	ro (	Cys \ 430	/al i	-ys
5	(2)	INFO	RMATI	ON F	OR S	EQ 1	D NO	: 34	:					-		
0		(i	(A) (B)	LEN TYP	CE CH GTH: E: an	464 nino	ami	no ad	CS: cids							
	(	(ii)			OLOGY TYPE											

		(×i	) SE	QUEI	ICE I	DESCI	RIPTI	OH:	SEQ	ID 1	40: i	34:				
	Мет -32		Ser -30		ı Val	111	∈ Gly	Th:		Thi	: Sei	r Gly	- 20		g Lys	s Val
5	Тул	-15		Ser	Leu	) Lei	յ Leu -](		- G] }	/ Phe	· Trp	Asp		s Val	l Thi	r Cys
	His 1	_	Ser	Pro	val e	Asp	o lle	сув	Thr	Ala 10		: Pro	Arq	g Asp	) Ile	≘ Pro
10	Met	Asn	Pro	Met 20		Ile	Tyr	Аrg	Ser 25		61u	Lys	Lys	Ala 30		Glu
	Asp	Glu	Gly 35		G] u	Glr	Lys	Il∈ 40		Glu	Ala	Thr	Asr 45		a Arç	, Val
15	Trp	Glu 50		Ser	Lys	Ala	Asn 55		Arg	Phe	Ala	Thr 60		Phe	: Туг	Gln
	His 65		Ala	Asp	Ser	Lys 70		Asp	Asn	Asp	Asn 75		Phe	Leu	Ser	Pro 80
20	Leu	Ser	Ile	Ser	Thr 85		F'he	A.l a	Met	Th: 90	-	Leu	Gly	Ala	. Cys 95	Asn
	Asp	Thr	Leu	Gln 100		Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110		Ser
25	Glu	Lys	Thr 115	Ser	Asp	Gin	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125		Asn	Cys
	Arg	Leu 130	Туг	Gln	Lys	Ala	Asn 135	Γλ.ε	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
30	Arg 145	Leu	Phe	ely	Asp	Lys 150	Ser	Leu	Thr	Ph∈	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Туг	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Г'nŧ
35	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190		Asn
	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
40	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Туг 220	Phe	Lys	Gly	Leu
	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
45	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
	Lys	Phe	-	Туг 260	Àrg	Ārģ	٧al		Glu 265	Gly	The	Gln	Val	Leu 270	Glu	Leu
50	Pro	Phe	Ly's 275	Gly	<i>F</i> .sp	Asp	lle	Thr 280	Met	Val	Leu	lle	Leu 285	Pro	Lys	Pro
	Glu	Lys	Ser	Leu	Ala	Lуs	Val	Gl u	Lys	Glu	Leu	Thr	Pro	Glu	Val	Leu

		290					29	5				360				
õ	Glr 305	Glu	Trp	Leu	Asp	Glu 310	ı Le	⊔ Glu	ı Glu	u Met	Met 315	Leu	Val	Va l	His	ме 32
-	Pro	Arg	Phe	Arg	11e 325	Glu	ı Ası	o G1?	/ Phe	Ser 330	Leu	Lys	Glu	Gln	Le:	
10	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350		Pr
	Gly	Ile	Val 355	Ala	Glu	Gly	Arç	360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
15	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
75	Ser 385	Thr	Ala	Val	Val	Ile 390	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
20	Leu	Asn	Thr	Ile 420	Ile	Phe	Met	Gly	Arg 425	Val	Ala .	Asn	Pro	Cys 430	Val	Lys
25	(2)	INFO	i) SI (A)	EQUE LEI	NCE NGTH	CHAF	RACTI	ERIST	rics:	!s						
30		(ii) (xi)	(D)	TOI	POLO E TY	GY: PE:	line prot	ear ein	EO I	D NO	: 35:					
35	Met '	Tyr S	Ser A -30	sn V	/al :	Ile	Gly	Thr -25	Val '	Thr S	Ser G	Sly I	20			
							-10					-5 .			•	
40	His C				3					10					15	
	Met A	** .	. '	٠,					25					30 .	٠.	
15	Asp G		-					40				•	45			
	Trp G	lu L. 50	eu Se	r Ly	/s A	la A	sn 5 55	er A	rg P	he A	la Th	nr Ti 50	nr Pi	he T	yr G	ln
	His L 65	eu Al	la As	p Se	r L	ys A 70	sn A	sp A	sn A	sp As	sn Il 75	e Ph	e Le	eu S		ro 80
0	Leu S	er Il	.e Se	r Th 8	r A. 5	la Pi	he A	la M	et Ti	hr L) 90	s Le	u Gl	y Al		ys A 95	sn

		Asp	o The	Leu	10(		Leu	ı Mei	E G)	u Va 10		e Ly	s Ph	e Asj	o Th. 12:		e Ser
5		Glu	ı Lys	Thr 115		Asp	Glr.	. Ile	= H1: 120		e Ph	e Ph	€ Ala	a Lys 125		u Asi	n Cys
3		Arg	130	_	Arg	Asn	Ala	: Asr 135	_	s Se	r Se	г Бу	146		l Se:	r Ala	a Asn
		Arg 145		Phe	ej y	Asp	Lys 150		Lei	Th.	r Pho	8 Asi 155		Thi	Ty	r Gli	n Asp 160
10		Ile	Ser	Glu	Leu	Val 165	-	G] 7	Ala	Ly:	5 Leu 17(		Pro	) Leu	н Дер	Phe 175	E Lys
		Glu	Asn	Аla	Glu 180	Gln	Ser	Arg	Ala	18:		e Asr	Lys	Trp	Va)		Asn
15		Lys	Thr	Glu 195		Arg	Ile	Thr	Asp 200		lle	Prc	5er	Glu 205		Ile	: Asn
		Glu	Leu 210	Thr	Val	Leu	Val	Leu 215		Æsr	The	Ile	Туг 220		Lys	G12	' Leu
20		Trp 225	_	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235		Glu	Leu	Phe	Tyr 240
		Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250		Met	Tyr	Gln	Glu 255	Gly
25		Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	-	Thr	Gln	Val	Leu 270		Leu
		Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
30		Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Ly's	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
		Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Vāl	His	Мет 320
35		Pro	Arg	Phe	Arg	Ile 325	Сĵп	Asp	GJ7.	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
,	•	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
40		Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
		His	Lys 370	Ala	Phe	Leu		Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Æla	Ala	Ala
45		5er 385	Thr	Ala	Val		11e 350	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
		Thr	Phe	Lys .		Asn . 405	Arg	Pro	Phe	Leu	Val 410	Phe	Il∈	Arg	Glu	Val 415	Pro
50		Leu	Asn		11e 420	Ile	Phe !	Met	Gly	Arg 425	Val	Ala	Asn		Cys 430	Val	Lys

	(2	) IN:	FORM	OITA	N FO	R SE	QID	NO:	36:							
5				(A) :	LENG' TYPE	E CHA TH: ' : am: LOGY	464 . ino :	amin acid	o ac	S: ids						
		(ii	L) MO	DLEC	JLE :	TYPE	: pro	otei	n							
10	36.					DESC										
	- 32	ryi 2	-30	Asr	n Va.	! Ile	e G17	7 Thi	r Val	l Th:	r Se	c G1;	y Lys -20		g Lys	s Val
15	Туг	Leu -15	Lei	ı Ser	Let	ı Lev	Leu -10	ı Ile	e G1:	y Phe	Trp	Asp -5		s Vai	l Thi	Cys
,,,	His	Gly	/ Ser	Pro	Val	l Asp	. Il€	Cys	Th:	Ala 10		s Pro	Arç	Ası	) Ile 15	Pro
	Met	: Asn	Pro	Met 20	Cys	: Ile	Туг	Arç	3 Sei 25		Glı	Lys	Lys	Ala 30		Glu
20	Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	11e 40	Pro	Glu	Ala	Thr	Asn 45		J Arg	, Val
0.5	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55		Arç	p Phe	: Ala	Thr 60		Phe	? Tyr	Gln
25	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	) Asr	Asp	Asn 75		Phe	Lei	Ser	Pro 80
•	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	A.la	Met	Th: 90		Leu	Gly	Ala	Cys 95	Asn
30	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105		Lys	Phe	Asp	Thr		Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120		Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
35	Arg	Leu 130	Tyr	Arg	Gln	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
10	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
40	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	qsA	Phe 175	Lys
	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn
45	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
50	Trp 225	Lys	Ser	Lys	Phe	ser 230	Pro	Glu	Asn		Arg 235	Lys	Glu	Leu	Phe	Tyr 240

	Ly	s Al	a As	¢ €37	/ G1 u 245		Cys	Se:	r Ala	5e1 250		1461	ту	r 61n	61 25	u Gly 5
5	Ly	s Ph	e Ar	7 Ty1 260	_	Arg	Val	. Ala	Glu 265	_	Thr	Glr	o Vā.	l Leu 270		ı Let
	Pr	o Ph	e Ly: 27:		/ Asp	Asp	llè	Thr 280		Val	Leu	11e	Let 285		Ly:	e Pro
10	G1	u Ly 29		Leu	Ala	Lys	Val 295		Lys	Glu	Leu	Thr 300		o Glu	Val	L Leu
	Gl:		u Trp	Leu	Asp	310		Glu	Glu	Met	Мет 315	Leu	Va)	Val	His	Met 320
15	Pro	o Are	g Phe	Arg	Ile 325	Glu	Asp	G] À	Phe	Ser 330	Leu	Lys	Gl u	ı Gln	Leu 335	Gln
	Asp	o Met	t Gly	Leu 340	Val	Æsр	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
20	G1	/ Ile	¥ Val 355		Glu	СΊУ	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365		Ala	Phe
	His	Lys 370		Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
25	Ser 385		: Ala	Vāl	Val	11e 390	Ph∈	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
,	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Ph€	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
30	Leu	Asn	Thr	Ile 420	Ile	Phe	Met	Gly	Arg 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys
35	(2)	INF	ORMA!	NOIT	FOR	SEQ	ID N	0: 3	7:							
50			() ()	SEQUE A) LE B) TY D) TO	NGTH PE:	: 46 amin	4 am	ino id		s						
40			) MOI				•									
							Gly '	Thr					Lys	Arg :	Lyrs	Val
<b>1</b> 5	-32 Tyr		-30 Leu	Ser	Leu 1		Leu :	-25 Il∈	Gly 1	Phe :	Prp A	sp	-20 Cys	Val :	<b>r</b> hr	C7.s
	His 1	-15 Gly	Ser	Pro '	Val 2 5		-10 Ile (	Cys :	Thr 2	Ala 1 10	ב אינ.	-5 Pro 2	Arg.	Asp 1	ile 15	Pro
50	_	Asn	Pro	Met (		lle T	ryr A	arg :	5er B 25		Slu I	ys 1	Lys .	Ala T 30		Glu

	As	p Gl	u G1 3	у Se 5	r Gl	u Gl	n Ly	s Il 4	e Pr O	o Gl	u Al	a Th		n Ar 5	g Ar	g Val
5	Tr	p G1 5	u Le	u Se	r Ly	s Al	a Asi	n Se S	r Ar	g Ph	e Al		r Th O	r Ph	е Ту	r Glr
	Hi 6	s Le 5	u Al	a As	p Se	r Ly	s Ası	n As	o As	n As	p A.s 7		e Ph	e Le	u Se	r Pro
10	Le	u Se	r Il	e Se	r Th:	r Ala	a Phe	e Al:	э Ме	t Th.		s Le	u Gl	y Al	a Cy:	s Asn
	As	p Th	r Le	u Gl:	n Gli	n Lei	⊔ Met	Glu	10:		e Ly:	s Pho	e Ası	P Th 11		≥ Ser
15	Gl	u Ly:	s Th	r Se:	r Asp	o Glr	ı Ile	His 120		e Phe	e Ph∈	≥ Ala	a Ly:		u Ası	n Cys
	Gli	n Lei 130	и Ту: О	r Arg	g Lys	s Ala	Asr 135	Lys	Se:	r Sei	Lys	140		l Se.	r Ala	Asn
20	Arc 145	J Let	ı Phe	e Gly	y Asp	150	s Ser	Leu	Thi	r Phe	Asr 155		Thi	ту	r Glr	Asp 160
	Ile	e S∈i	Glı	ı Lev	1 Val 165	Tyr	Gly	Ala	Lys	170		Pro	Lei	a Asp	Phe 175	Lys
25	Glu	ı Asr	n Ala	180	Gln	ser	Arg	Ala	Ala 185	ı Ile	: Asr	Lys	Trp	Val 190		Asn
	Lys	Thr	Glv 195	Gly	' Arg	Ile	Thr	Asp 200	Val	. Ile	Pro	Ser	Glu 205		a Ile	Asn
30	Glu	Leu 210	Thr	· Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220		Lys	Gly	Leu
	225					230					235					Tyr 240
35	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270	Glu	Leu
0			275					280					285		Lys	
		290					295					300		. *	Val	
5	305			•		310					315				His	Met 320
					325					330					Leu 335	
o				340					345					350	Leu	
	Gly	Ile	Val 355	Ala	Glu	Gly	Arg .	Asp . 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe

	ні	s Ly 37		a Ph	e Le	u G).	u Va 37		in Gl	.u GÌ	u Gl	y 5e		u Al	.a Al	a Al.
5	Se. 38.		r Al	a Va	l Val	1 11 39		e Pr	o Ar	g Se	r Le 39		n Pr	o As	n Ar	g Va. 40
	Th.	r Ph	e Ly.	s ¥J	a Ası 403		g Pr	o Ph	e Le	u Va 41		e Il	e Ar	9 Gl	υ Vā 4]	1 Pro 5
10	Lev	u Ası	n Th	r Il 42		€ Pho	≘ Ме	t Gl	y Ar 42		) A.)	a As	n Pr	c Cy 43		l Lys
	(2)	INI	FORM	ATI OI	ı FOF	SEÇ	O I D	ио:	38:							
75			(	(A) 1 (B) 7	JENCE LENGT TYPE: TOPOL	H: 4 ami	64 a	amin ocid								
		(ii	) MC	LECU	LE T	YPE:	pro	tei	מ							
20	Met				Vel								7 7.175	. Lro	7 I.Ve	. Val
	-32		-30	1				-25	ò				-20	)		
25	Tyr	Leu -15		Ser	Leu	Leu	Leu -10		: G]/	, Phe	Trp	2.sp -5		: Val	. Thr	C7.2
	His 1		Ser	Pro	Val 5	Asp	Ile	Cys	Thr	Ala 10	_	Pro	Arg	Asp	Ile 15	Pro
0 .	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Arg	Ser 25		Glu	Lys	Lys	Ala 30		Glu
	Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	Ile 40		GГл	Ala	Thr	Asn 45	-	Arg	Val
5	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Ph∈	Ala	Thr 60	Thr	Phe	Tyr	Gln
	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	lle	Phe	Leu	S∈r	Pro 80
0	Leu	Ser	Ile	Sei	Thr 85	Ala	Ph∈	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
	Asp	Thr		Gln 100		.Leu	Met		Val 105		Lys	Phe	A.sp	Thr 110	Ile	Ser
5	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cha.
5	Glņ	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	Gly		Lys 150	S∈r.	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
)	Ile	Ser	Glu	Leu	Val 165	Tyr	ely	Ala	Lys	Leυ 170	Gln	Pro	Leu	Asp	Phe 175	Ly's

	Glu	Asn	Ala	Gl u 190	Gln	) Ser	: Arç	Ala	Ala 195		e Asr	ı Lys	s Trp	o Val		r Asn
5	Lys	Thr	Glu 195	Gly	Arg	I'e	Thr	Asp 200	Val	Ile	e Pro	Sei	Glu 205		116	e Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215		Asn	Thi	Ile	Tyr 220		e Lys	Gly	/ Leu
10	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235		Glu	ı Leu	Phe	Tyr 240
	ъys	Ala	qzA	Glý	Glu 245	Ser	Суѕ	Ser	Ala	Ser 250		Met	Tyr	Gln	Glu 255	Gly
15	Lys	Phe	Arg	Туг 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Va <u>l</u>	Leu 270		Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285		Lys	Pro
20	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300		Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	M∈τ 315	Leu	Val	Val	His	Met 320
25	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	2he	Ser 345	Pro	Glu	Lyrs	Ser	Lys 350	Leu	Pro
30	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Alá	Phe	Leu	Glu	Val 375	Asn	Glu	Gl u	Gly	Ser 380	Glu	Ala	Ala	Ala
35	Ser 385	Thr	Ala	Val	Val	Ile 390	Val	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys	Ala.	Asn 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
40	Leu	Asn	Thr	Ile : 420	Ile	Phe	Met		Arg 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys
. 45	(2)	INFO	RMAT	ION !	FOR	SEQ	ID N	O: 3	9:							
40		(:	(A (B)	EQUEI LEI TYI TOI	NGTH PE: a	: 46 amin	4 am:	ino a id		s						
50		(ii)				•										
		(xi)	SEQU	JENCE	DES	SCRI	10 I T	1: SE	Q II	ON O	39:	:				

	Ме t - 33		- 361 - 30		ı Val	1 116	e G1;	y Tr. -2		l Th	r Se	r Gl	y Ly -2		ց Իչ	s Val
5	Tyrz	: Leu -15		ı Sei	: Leu	ı Lêt	- let		ė (3)	y Ph	e Tr	ր Դ.s. 	_	s Val	l Th	r Cys
	His 1		Ser	Fro	, Va <u>)</u> 5		: 11e	е Су	s Th	r Al l		s Pr	o Are	g Asp	) 11e	÷ Pro 5
10	Met	Asn	Pro	Мет 20	_	: 11e	Tyr	Arq	9 Se. 23		o Gli	יעב ני	b Lys	30		Glu
	Asp	Glu	Gly 35		Glu	Gln	Lуs	3 Ile 40		o Gli	: Ala	5 Thi	Ast 45	-	Arç	y Val
15	Trp	Glu 50		Ser	Lys	Ala	Asn 55		Ar q	g Phe	: Ala	Thr EC		Phe	туг	Gln
	His 65		Ala	Asp	Ser	Lys 70		Asp	Asr	Asp	75 Asr		Phe	Leu	Ser	Pro 80
20	Leu	Ser	Il∈	S∈r	Thr 85	Æ.l≥	Phe	Älā	Met	Thr 90		Leu	Gly	Ala	Cys 95	Asn
	Asp	Thr	Leu	Gl n 100	Gln	Leu	Met	Glu	Val 105		Lys	Phe	Asp	Thr 110	Ile	Ser
25			115					120					125			C7.≥
	Gln	Leu 130	Tyr	Arg	Lys ·	Ala	Asn 135	Lys	Ser	Ser	īys	Leu 140	Val	Ser	Ala	Asn
30	Arg 145	Leu	Phe	Gly	Asp	Lys 150	S∈r	Leu	Thr	Phe	Asn 155	Glu	Thr	Туг	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
35				180		Ser			185			-	•	190		
			195	_	_	Ile		200					205			
40		210					215					220		-	-	
	225					Ser 230					235					240
45 .					245	Ser.				250					255	-
				260	_	Arg			265					270		
50			275		-	Asp :		280					285		-	
•	Glu	ьуs . 290	ser.	⊾eu.	mia .		382 Val	e≟U	тЛS	ein		7hr 300	PIO ·	เป็น ,	val	Leu

	G) 30	ln Gl O5	lu T	rp L	eu A	sp G	51 u 51 0	Leu	61	u G1	u Me	et Me	t 1	eu V	āl V	al H	15	Ме 32
5	Pi	ro Ai	g Pi	ne A	rg I. 32	le G 25	ilu	Asp	Gl	y Ph	€ S€ 33	r Le	u L	ys G	lu G		eu 35	Gl
	As	яр Ме	t G	ly Le 34	eu Va 10	el A	.sp	Leu	Phe	34	r P: 5	o G1	u L	ys Se		ys Lo	eu	Pr
10	Gl	y Il	e Va 35	al Al 55	a Gl	Lu G	ly .	Arg	Asr 360	As <sub>l</sub>	p Le	и Ту	r Va		er A.s 55	sp Al	la	₽h
	Hi	s Ly 37	s Al	a Ph	e Le	eu G	lu Y	Val 375	Asn	Gli	ı GJ	u Gl	у Se 38		lu Al	ā Al	la.	Al
15	Se 38	r Th	r Al	a Va	l Va	1 A 3	la 1 90	Leu	Gly	Arg	g Se	r Le 39	u As 5	n Pr	o As	n Ar		Va:
	Th	r Ph	e Ly	s Al	a As 40	n A.	rg !	Pro	Phe	Let	1 Va. 41	1 Ph 0	e Il	e Ar	g G1	บ Va 41		Pro
20	Le	u As:	n Th	r Il 42	e Il O	e Pì	ne M	4et	Gly	Arg 425	y Vai	l Ala	a As	n Pr	o Cy 43		1 1	Lys
	(2	) IN	FORM	ATIO:	N FO	R SE	EQ I	ID N	10:	40:								
25				(A) (B)	UENC LENG TYPE TOPO	Тн: : ап	464 ni no	: ап	nino :id	TICS aci	: ds							
					JLE '													
30	Met -32	Tyr	Sei	: Asr	NCE I			ly '	Thr					/ Ly:	s Arq	J Ly:	s V	'al
35		Leu -15	-30		: Let	ı Le	u L		-25 Ile	Gly	Phe	Trp	Asp	-20 Cys		. Thi	r C	ys
	His 1	Gly	Ser	Pro	Val 5	. Ası			Cys	Thr	Ala 10	Ile			J Ser	: Ile		ro
40	Met	Asn	Pro	Met 20	Cys	Ile	∍ T\	yr A	Arg	Ser 25	Pro	Glu	Lys	Lys	Ala 30		G.	lu
	Asp	Glu	Gly 35	Ser	Glu	Glr	ı Ly	/s I	1e 40	Pro	Glu	Alą	Thr	Asn 45	Arg	Arg	Va	21
45	Trp	Glu 50	Leu	Ser	Lys	Ala	As 5	n S	Ger .	Arg	Phe	Ala	Thr 60	Thr	Phe	туг	G)	מו
	His 65	Leu	Ala	Asp	Ser	Lys 70	As	n A	sp )	Asn.	Asp	Asn 75	lle	Phe	Leu	Ser		0
50	Leu	Ser	Ile	Ser	Thr 85	Ala	Ph	e A.	la M	1et 1	Thr 90	Lys	Leu	Gly	Ala	Cys 95	As	n
	Asp	Thr	Leu	Gln 100	Gln	Leu	Мe	t G	1 u \	/al 1	Phe	Lys	Phe	Asp	Thr 110	Ile	Se	r

	Glu	Ъ'nг	Thr 115		: Asp	. Glr	, Ile	: Hi:		e Ph	e Phe	÷ A1:	5 Lys 125		ı Ası	a Cže
5	Arg	Leu 130	_	Arç	r Lys	Ala	: Asn 135	_	s Se.	s Se.	r Lys	Let 14(		l Sei	: Ala	a Asn
	Arg 145		Phe	G1 y	Asp	Lys 150		Let	Th:	r Phe	255		Th:	г Туг	Glr	160
10	Ilė	Ser	G] ນ	L÷u	Val 165	_	Gly	Ala	Lys	170		Prc	) Let	) Asp	Phe 175	. Lys
	Glu	Asr.	Alā	Glu 180		Ser	Arg	АÌа	Ala 185		e Asn	Lys	Trp	Val 190		Asn
15	ъуs	Thr	Gl u 195	_	Arg	Il∈	Thr	Asp 200		Ile	: Pro	Ser	Glu 205		Il∈	: Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	A.s.ri	Thr	Ile	Туг 220		Lys	Gly	рел
20	Trp 225	īys	Ser	Lys	Ph∈	Ser 230		Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Туг 240
	Lys	Ala	Asp	СJА	Glu 245	Ser	Суѕ	Ser	Ala	Ser 250		Met	Tyr	Gln	Gl u 255	Gly
25	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gl y	Thr	Gln	Val	Leu 270	Glu	Leu
	Pro	Phe	Lys 275	Gly	Æsp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys ·	Pro
30	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
35	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
40	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	L∈u	Туг	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	L∈u	Glu	Val 375	Asn	Glu	Glu	GŢλ	Ser 380	Glu	Ala	Ala	Ala
45	Ser 385	Thr	Ala	Val		11∈ 390	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
50	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
50	Leu	Asn		Ile 420	Ile	Phe	Met Met	Gly	Arg 425	Val	Ala.	Asn	Pro	Суs 430	Val	LУs

	(2	!) IN	FORM	ATIC	n Fo	OR SE	II Q	HO:	: 41	:							
5			(i)	(A) (B)	LENG TYPE	E CH STH: C: aπ OLOGY	464 Nino	amir acio	ns er E	CS: cids							
						TYPE											
											NO:						
10	Me - 3	t Ty: 2	r Se -3	r As: O	n Va	1 11	e Gl	у Тh -2	r Va 5	el Th	ır Se	r Gl	у Ly -2		g Ly	s Val	
	Ту	r Let -15	ı Le	u Se:	r Le	u Le	u Le -1	u Il O	e Gl	y Ph	e Tr	p As	р Су 5	s Va	l Th	r Cys	
15	His	s Gly l	y Se	r Pro	o Va	l As <sub>l</sub> 5	p Il	e Cy	s Th		a Il O	e Pr	o Ar	g Se		e Pro 5	,
	Met	: Asr	n Pro	Met 20	: Су. )	s Ile	е Ту.	r Ar	g Se 2		o Gl	ı Ly:	s Ly	s Ala 30		r Glu	
20	Asp	Glu	35	, Ser	Gl	ı Glı	ı Lys	s Il 4	e Pr O	o Gl	u Ala	Th:	r A.sı 4		g Ar	g Val	
	Trp	61u 50	Leu	ser	Lys	s Ala	Asr 55	s Se	r Ar	g Ph	e Ala	Th:		r Phe	э Ту.	r Gln	
25	His 65	Leu	Ala	Asp	Sei	Lys 70	Asr	n Asp	o As	n Ası	Asr 75		Ph€	e Leu	ı Se	r Pro 80	
	Leu	Ser	Ile	Ser	Th:	: Ala	Phe	e Ala	a Mei	t Th:	r Lys	Leu	Gl	/ Ala	Cys 95	s Asn	
30	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Va]	L Phe	: Lys	Phe	Asp	Thr		e Ser	
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Ph∈	: Phe	Ala	Lys 125		Asn	Cys	
35	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn	
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160	
40	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175		
	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys <sub>.</sub>	Trp	Val 190	Ser	Asn.	
45	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn	
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu	
50	Trp 225	Lys .	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240	

	Lys	Ala	Asp	e3 7	Glu 245	Ser	Cys	Ser	Ala	Ser 250		Met	Ŧy:	6ln	Gl u 255	Gly
5	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	A.la	G) u 265	61 y	Thr	Gln	Va]	L∈ս 270	Glu	Lēu
	Pro	Ph∈	Lys 275	Gly	Asp	Asp	lle	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lյ <sub>ʻ</sub> ε	Pro
10	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	глг	Glu	Lēu	Thr 300	Fro	Glu	Val	Leu
	Gln 305	Glu	Trp	Lēu	Asp	Glu 310	Leu	Glu	Glu	Мет	Met 315	Leu	۷āl	Val	His	Met 320
15	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	Gly	Leu 340	Vāl	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Sēr	Lys 350	L∈u	Pro
20	Gly	Ile	Val : 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr		Ser 365	Asp .	Ala	Phe
	His	Lys . 370	Ala :	Ph∈	Leu (	Gl u	Val 375	Asn	Glu (	eja (		Ser ( 380	3lu	Ala A	Ala 1	4.1 a
25	Ser ' 385	Thr i	Ala V	/al '	Vāl :	11e 390	Val	Pro .	Arg S		Leu <i>)</i> 395	Asn I	ro.	Asn A	-	/al 400
	Thr	Phe 1	Lys 2	la ?	isn . 405	arg :	Pro :	Phe :		/al E	Phe I	le A	rg (	Glu \ 4	/al i	Pro
30	Leu A	Asn ]	Thr I	le 1 20	[le F	he i	viet (		Arg V 125	/al /	Ala A	sn P		Cys V 130	al I	ys
	(2) I	NFOF	MAT I	ON F	or s	EQ I	ם אכ	): 42	: :							
35			) SE (A) (E)	QUEN LEN TYF	ICE C	HARA 464 mino	CTEF ami	RISTI .no a	CS:							
			MOLE			•										
10	Met T	yr S	SEQUE er As				1ут	hr V						rg L)	ıs Va	ì
	-32 Tyr L		30 ≘u S∈	r L	≧u Le	eu L		25 le G	ly Ph	ne Ti				≥l Th	ır Cy	; <b>5</b>
	His G		er Pr	o Va	al As			s Th		.a Il		o Ar	g Se		e Fr 5	0
	Met As	sn Pr	o Me 2	τ C)	s Il	е Ту	r Ai	:g Se			u Ly	s Ly.				บ
50	Asp Gl	.u Gl 3	չ՝ Տ≘ :5	r Gl	u Gl	u P?		e Fr 0	c Gl	u Al	a Th	r A.sı 4!	n Ar		g Va	1

101

	Tr	p G1 5	u Le O	u Se	r Ly	s A.l	a A.s 5	n Se S	r Ar	g Fh	∈ A <u>l</u>	а 7'h б		r Ph	е Ту	r Gl:
5	Ні 6	s Le 5	u Al	a As	p Se	r Ly.	s As: 0	n As	p As	n As	p Ası 7.		e Ph	e Le	u Se	r Pre
	Le	u Se	r Il	e Se:	r Th.	r Ala	a Ph	e Al	a Me	t Th		s Le	u Gl	y Al	a Cy 9	s Ası 5
10	Ası	P Th	r Lei	u Glr 100	n Gli	n Lei	u Mei	c Gl	u Va. 10:		e Lys	s Pho	e As	p Th 11		e Sei
	Glı	ı Ly:	Th:	s Ser	r Asp	o Glr	n Ile	P Hi:	s Pho	e Phe	≘ Ph∈	e Ala	a Ly:		u As:	n Cys
15	Arg	130	а Туг )	r Arg	g Lys	a Ala	2 Asr 135		s Se	r Sei	r Lys	Let 140		l Se	r Ala	a Asr
	Arc 145	Let	ı Phe	e Gly	/ Asp	150	Ser	Lei	Th:	r Phe	e Asr 155		ı Thi	т Ту.	r Gli	n Asp 160
20	Ile	: Sei	Glu	ı Leu	Val 165	Туг	: Gly	, Ala	a Lys	170		Pro	Leu	ı Ası	⊃ Ph∈ 175	e Lys
	Glu	Asr	Ala	Glu 180	Gln	Ser	Arg	Ala	a Ala 185	i Ile	: Asn	Lys	Trp	Val 190		: Asn
25	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200		Ile	Pro	Ser	Glu 205		3 Ile	e Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	. Asn	Thr	Ile	Тус 220		: Lys	s Gly	/ Leu
30	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235		Glu	Let	ı Phe	Tyr 240
	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly
35	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265		Thr	Gln	Val	Leu 270		Leu
	Pro	Phe	Lys 275	Gly	qzA	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
40	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
٠	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Vạl	His	Met 320
45	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
,0	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
50	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
50	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu		Ser 380	Glu	Ala	Ala	Ala

	Se 38		r Al	a Va	:i Va	:1 Al 35		tu G]	ly As	rg S	er Le 39		in P	rc As	en Ar	rg Va 40
5	Th	r Ph	e Ly	s 7.1	a As 40		g Fi	o Ph	ie Le		1 Ph	e Il	le Ai	rg Gl	u Va 4]	al Pro 15
	Le	u As	n Th	r Il 42		e Ph	e Me	et Gl	у Аг 42		:1 A1	a As	n Pi	10 Cy 43		il Lys
70	(2	) IN	FORM	atio	N FO	P. SE	O ID	но:	43:							
15				(A) (B)	LENG TYPE	E CHA	4.64 ino	amin acid	o āc							
.5		15.5				LOGY:			_							
						TYPE: DESCF	•			TD	NO · A	1 Q ·				
20	Met												v 1,02	s žro	7 T.V	s Vāl
	-32	?	-30	)				-25	5				-21	D		
	Tyr	Leu -15	Leu )	Sei	. Lei	ı Leu	l Let -1(		€ Gly	y Ph	€ Trp	Asp -5		s Val	l Th:	r Cys
25	His 1		' Ser	Pro	Val		ije	e Cys	Thi	r Ala 10		Pro	Arg	g Asp	) Ile	∍ Pro
	Met	Asn	Pro	Мет 20		Ile	Tyr	: Arg	Ser 25		Glu	Lys	Lys	30		: Glu
0	Asp	Glu	Gly 35		Glu	Gln	L)'s	11e 40		Glu	Ala	Thr	Asn 45	-	Arç	, Val
	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55		Arg	Phe	Ala	Thr 60		Phe	Туг	Gln
5	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 30	Lys	Leu	Gly	Ala	Cys 95	Asn
0	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Ph∈	Phe	Phe	Ala	Gln 125	Leu	Asn	C7.2
5	Arg	Leu 130	Tyr	Gln	Asn	Ala	Asn 135	Lys	Ser	Ser	Lys	L∈u 140	Val	5er	Ala	Asn
_	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
D	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Гλε

	Gli	ı Asr	n Ala	Gl u 180	Glr.	Ser	·Λις	g Ala	Ala 185	Ile	≥ Ası	Lys	Tr	o Val 190		. As!
5	Lys	Th:	Glu 195	Gly	Arg	Ile	Th:	200	Val	Ile	e Pro	Ser	Gl: 205	u Ala	a Ile	e As:
	Glu	1 Let 210	Thr	Val	Leu	Val	215	val	Asn	Th	Ile	Tyr 220		≞ Lys	s Gly	' Lev
10	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	The	Arg 235		Glu	l Let	Phe	Туг 240
	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	туг	Gln	Glu 255	
15	Lys	Phe `	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270		Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285		Lys	Pro
20	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
25	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	?he	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
30	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
35	Ser 385	Thr	Ala	Val	Val	Ile 390	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys .	Ala .	Asn . 405	Arg	Pro	Phe		Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
40	Leu	Asn	Thr :	lle :	Ile	Phe i	Met	Gly .	Arg 425	Val	Ala.	Asn	Pro	Cys 430	Val	Lys
	(2)	INFO:	RMATI	ON I	FOR S	SEQ :	ID N	0: 4	4:							
45		(:	(B)	LEN TYE	NGTH:		am ac.	ino a id		5						
		(ii)	MOLE	CULE	TYF	E: F	prote	ein								
50			SEQU													
	Met 7 -32	Tyr 5	Ser A -30	sn V	al I	le G	Sly 7	hr V 25	al T	hr s	Ser G		ys <i>?</i> 20	Arg !	Lys \	al

	Туз	Let -15		ı Se:	r Lei	) Lė(	1 Let -10		∈ G1	y Pr	ie Ti	p As	р С <u>у</u> 5	rs Va	1 Th	er Cys
	His 1	: G]7	, Ser	Pro	Val 3	Asp	, lla	e Cy.	s Th		a Ly	s Pr	c Ar	g As		e Pro 5
5	Мет	Asn	Pro	мет 20		Ile	туз	r Ar		r Fr 5	o Cl	υ <u>L</u> ;∙	s L;	s Al 3		r Glu
	Asp	Glu	Gly 35		Glu	Gln	Lys	: Il:		၀ Gl	u Al	a Th		n Ar 5	g Ar	g Val
70	Trp	Glu 50		Ser	Lys	Ala	Asn 55		r Ar	g Ph	e Al	= Тh. б(		r Ph	в Ту	r Gln
	His 65		Ælā	Asp	Ser	Lys 70	A.s n	Asp	) As	n As	p Asi 7:		e Ph	e Lei	ı Se.	r Pro 80
15	Leu	Ser	Il∈	Ser	Thr 85	Ala	Phe	Ala	Mei	t Th		s Lei	ı Gl	y Ala	5 Cy:	s Asn 5
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Va]		≞ Lys	Phe	e Asp	> Thi		e Ser
20	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120		e Phe	∋ Phe	Ala	Glr 125		Asr	Cys
	Ærg	Leu 130	Tyr	Gln	Asn	Ala.	Asn 135	Lys	Ser	: Ser	: Lys	Leu 140		. Ser	Ala	Asn
25	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	: Asn 155		Thi	Tyr	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	СŢЪ	Ala	Lys	Leu 170		Pro	Leu	Asp	Phe 175	Lys
	Glu	Asn	Ala	Glu. 180	Gln	Ser	Arg	Ala	Ala 185		Asn	Lys	Trp	Val 190		Asn
	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	S€r	Glu 205		Ile	Asn
5	Glu	Leu 210	Thr	Val	Leu		Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
	Trp 225	Lys	Ser	ŗ'ns		Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lуs	Glu	Leu	Phe	Tyr 240
0	Lys	Ala .	Asp		Glu : 245							Met		Gln		
	Lys	Phe .		Tyř. 260	Arg )	Arg '	Val .		Glu 265	ej y	Thr	Gln	Val	L∈u 270	Glu	Leu
5	Pro		Lys ( 275	Gly 2	Asp A	Asp :		Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Ly's	Pro
	Glu	Lys : 290	Ser 1	Leu A	Lla I		/al ( 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
9	Gln (	Glu ?	rp 1	Leu A		lu 1 :10	Jeu (	Glu	Glu	Met	Met 315	Leυ	Val	Val	His	Met 320

	Pr	o Ar	g Phe	≥ Aro	32!	e G11 5	u As	o 61 <u>9</u>	/ Phe	330		ı Ly:	s Glu	ı Glı	1 Leu 335	Gln
5	As	p Met	t Gly	/ Let 340		l As;	o Lei	u Phe	Se 345		o Glu	ı Ly:	s Sei	T Lys 350		Pro
	Gl	y Ile	e Val	Ala S	ı Glu	ı Gly	y Ar	g Asp 360		o Lei	туг	(Val	1 Sei 365		Ala	Phe
10	Hi:	s Lys 370	s Ala	Ph∈	e Leu	ı Glu	1 Val 375		Glu	ı Glı	ı Gly	' Sei 380		ı Ala	Ala	Ala
	Se:	r Thr	r Ala	val	. Val	11e 390		. Pro	Arg	Ser	Leu 395		n Pro	Asn	Arg	Val 400
15	The	r Phe	E Lys	Ala	Asr 405		) Pro	Phe	Leu	Val 410		Ile	e Arg	g Glu	Val 415	Pro
	Lei	ı Asn	Thr	1le 420		Phe	. Met	Gly	Arg 425		Ala	Asr	Pro	Cys 430		Lys
20	(2)	TNF	ORMA	<b>ホェ</b> しか	೯೧೨	SEO	ח ז ו	NO.	15.							
	(2)	714						ERIS								
			(	A) L	ENGT		64 a	mino								
25						OGY:										
		(ii	) MO	LECU	LE T	YPE:	pro	tein								
30			) SE													
30	Met -32	Tyr	Ser -30	Asn	Val	Ile	Gly	Thr -25	Väl	Thr	Ser	Gly	Lys -20	Arg	Lys	Val
	Tyr	Leu -15	Leu	Ser	Leu	Leu	Leu -10	Ile	Gly	Phe	Trp	Asp -5	Cys	Val	Thr	Cys
35	His 1		Ser	Pro	Val 5	Asp	Ile	Cys	Thr	Ala 10	Lys	Pro	Arg	Asp	Ile 15	Pro
	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Arg	Ser 25	Pro	Glu	Lys	Lys	Ala 30	Thr	Glu
40	Asp		Gly 35					11e 40		Glu			Asn 45		_	Val
	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60	Thr		Tyr	Gln
45	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	019 08
50	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
50	Asp	Thr	Leu	Gln 100	Gln	Leu	Met		Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser

	6 <u>3</u> 1	ı Lys	s Th:		: Asp	. Gl:	n Il	= Hi: 110		e Fh	e Ph	≥ Ala	E 61		u As	r. Cys
5	Ar	3 Leu 130		Glr	. Asn	A1 a	135		s Se	r Se	r Lys	14(		) Se	r Al	a Asn
	Arq 145		) Phe	e 617	Asp	Lys 150		: Lei	ı Th	r Phe	a Asr 155		2 Th	r Ty	r <b>G</b> l:	n Asp 160
10	Ile	: Ser	Glu	Leu	Val 165		ej?	, Als	: <u>-</u> -7.:	170		Pro	> Let	u Asi	2 Pho 17	€ Lys 5
	Glu	Asn	Ala	189 189		Ser	Arg	Ala	Ala 185		: Asn	Lys	Tr	o Val 190		r Asn
15	Lys	Thr	Glu 195		Arg	Ilê	Thr	Asp 200		llie	Pro	S∈r	205		i Ile	≥ Asn
	Glu	Leu 210		Val	Leu	Val	Leu 215		Asr	Thr	Ile	Tyr 220		€ Lys	e 17	'Leu
20	Trp 225	Lys	Ser	Lys	Phe	Ser 230		Glη	Asn	The	Arg 235	Lys	Glu	Leu		Tyr 240
	Lys	Ala	Asp	вĵу	Glu 245	Ser	Cys	Ser	Ala	Ser 250		Met	Tyr	Gln	Glu 255	Gly
25	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265		Thir	Gln	Val	Leu 270		Leu
23	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	PFO
	Glu	Lys 290	Ser	Ľeυ 	<u>Al</u> a	Lys	Val 295	Glu	Lys	Ģlυ	Leu	Thr 300	Pro	Glu	Val	Leu
30	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
	Pro	Arg	Ph∈	Arg	11∈ 325	Glu	Asp	GJ 7.	Ph∈	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
35	Asp	Met	Gly	Leu 340	Val	Asp.	Leu	Phe	S∈r 345	Pro	Glu	Ly's	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
40	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glv	Glu		Ser 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Æla	Val	Val.	Ala 390	Leu	Gly.	Arg	Ser	Leu . 395	Asn	Pro	Asn	Arg	Val 400
45	Thr	Phe	Lys		Asn 1 405	Arg	Pro	Phe		Val 410	Phe	lle.	Arg	Glu	Val 415	Pro
	Leu	Asn		11e : 420	Ile 1	Phe	Met		Arg 425	Val.	Ala i	Asn.		Cys 430	Val	Lys
50																

(2) INFORMATION FOR SEQ ID NO: 46:

				SEQ (A) (B) (D)	LENG TYPE	TH: : am	464 ino	amin acid	o ac	:S: :ids						
5			i) M													
		( x	i) S	EQUE	ИСЕ	DESC	RIPT	ION:	SEQ	ID	ио:	46:				
10	Me -3	t Ty. 2	r Se.	r Ası	n Va.	1 11	e Gl	y Th -2	r Va 5	l Th	r Se	r Gl	y Ly -2		g Ly	s Val
	Ty	r Let	ı Let	ı Se:	r Le	u Le	u Le:	u Il O	e Gl	y Ph	e Tri	Ası		s Vai	l Th	r Cys
15	Hi	s Gly 1	y Sei	r Pro	o Vai	l Asp 5	o Ile	e Cy.	s Th	r Ala		s Pro	Ar	g As;	> Il	e Pro 5
	Me	t Asr	n Pro	Met 20	Cys	s Ile	≘ Туг	r Ar	g Se. 2:	r Pro	o Glu	ı Lys	s Lys	Ala 30		r Glu
20	Asp	o Glu	35	y Ser	Gli	ı Glr	l Lys	1 1 e	e Pro	o Glu	ı Ala	Thi	Asr 45		Arq	g Val
	Trp	50 50	Leu )	Ser	: Lys	Ala	Asn 55	ı S∈ı	r Arg	g Phe	e Ala	Thr 60		Phe	тул	c Gln
25	His 65	Leu i	Ala	Asp	Ser	T Lys	Asn	Asp	Asr	a Asp	Asn 75		: Ph∈	Leu	S∈1	Pro 80
	Let	ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90		Leu	G13	, Ala	Сус 95	Asn S
3 <i>0</i>	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110		Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125		Asn	Cys
35 .	Gln	Leu 130	Tyr	Gln	Asn	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn.
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
40	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg		Ala 185					Val 190		Asn
45	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Туг 220	Phe	Lys	Gly	Leu
	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
5 <b>0</b>	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr		Glu 255	Gly

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with carte and consideration

	ГÀг	Phe	Arg	Tyr 260		Arg	Va)	1 2.1 a	61 t 265		The	G) r	val	Leu 270		. Ն≙
5	Pro	Phe	Lys 275	G1 y	Asp	Asp	Ile	Thr 280	Met	(Vā)	. Leu	Ile	Leu 285		ЬУS	Pr
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295		Lys	Glu	Leu	Thr 300		Glu	۷æl	Lē
10	Gln 305	Glu	Trp	Leu	Asp	Glu 310		Glu	Glu	Met	Met 315	Lēu	Val	Val	His	Me: 32
	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	ely	Ph∈	Ser 330		Lys	Glи	Gln	Leu 335	
15	Asp	Met	Gl y	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	el'n	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
20	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val		Il∈ 390	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	V≥1 400
25	Thr	Phe	Lys .	Ala.	Asn . 405	Arg	Pro	Phe	L∈u	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
	Leu	Asn '	Thr	lle : 420	Ile	Phe :	Met	Gly	Arg 425	Val	Ala.	Asn		Cys 430	Val	Lys
30	(2)	INFO	PMA.T :	1011	FOR S	SEQ :	ID N	O: 4	7:							
35		(:	(B)	LEN TYP	VGTH:	CHAP : 46 amin GY: 1	am ac	ino id		S						
		(ii)				-										
10	Met : -32	(xi) Tyr S -					ily :					;1; <sup>,</sup> 1	∟γ's A -20	Arg I	Jys \	Val
	Tyr 1	L∈u l -15	eu S	er L	eu L		eu 1	lle (	Sly F	Phe T	rp A	.sp (	Cys ∖	/al J	'nr (	]/s
<b>1</b> 5	His O	Ely S	er P	ro V	al A 5	sp I	le C	Sys T	hr A	ala I 10	ys P	ro Æ	.rg Æ	sp I	le 9 15	ro
	Met A	esn P	ro M	et C 20	ys I	le T	yr A	irg S	er P 25	ro G	lu L	ys L	ys A	la T 30	hr G	11u
50	Asp G		ly 5 35	er G	lu G	ln L		le P 40	ro G	lu A	la T		sn A 45	rg A	rg V	al

		Т	p Gl 5	u Le O	u Se	r Ly.	s Al		n Se 5	r Ar	g Ph	e Al	a Th		r Ph	е Ту	r Gln
	5	Ні 6	s Le 5	u Al	a As	p Se	r Ly. 7	s As O	n As	p As	n As	p As:		e Ph	e Le	u Se	r Pro 80
		Le	u Se	r Il	e Se:	Th:	r Ala	a Ph	e Al	a Me	t Th		s Le	u Gl	y Al	а Су 9	s Asn 5
	10	As	p Th	r Le	u Glr 100	n Glr	ı Lei	u Me	t Gl	u Va 10	1 Ph	e Ly:	s Ph	e As	p Th 11		e Ser
		G1	u Ly.	5 Th:	s Sei	Asp	Glr	ı Ile	e Hi.		e Phe	e Ph∈	≥ Ala	a Gl 12		u As:	n Cys
	15	Gl	n Lei 130	а Туг Э	r Glr	Asn	Ala	13:		s Se	r Sei	Lys	14(		l Se	r Ala	a Asn
		Ar 145	g Let	Phe	e Gly	/ Asp	Lys 150	Se)	r Lei	u Th.	r Phe	Asr 155		Th.	r Ty	r Gli	qaA n 031
	20	Ile	e Sei	Glı	ı Leu	Val 165	Tyr	: Gl)	/ Ala	a Lys	170		Pro	Le:	As;	Phe 175	≥ Lys
		Glı	ı Asr	ı Ala	Glu 180	Gln	Ser	Arç	, Ala	185		Asn	Lys	Tr	0 Va]		Asn
2	25	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	200	o Va]	Ile	Pro	Ser	Gl: 205		ılle	e Asn
		Glu	210	Thr	Val	Leu	Val	Leu 215	val	. Asr	Thr	Ile	Tyr 220		: Lys	Gly	/ Leu
3	30	225				-	230				Thr	235					240
						245					Ser 250					255	-
3	35				260					265					270		
				2/5					280		Val			285			
40	10		290					295			Glu		300				
		305					310				Met	315					320
4	25					325					Ser 330					335	
					340					345	Pro				350		
5	0			222					360		Leu			365			
50		His	Lys 370	Ala	Phe :	Leu (	Glu	Val 375	Asn	Glu	Glu		Ser 380	Glu.	Ala	Ala	Ala

	S∈ 38		r Al	a Va	) Va	1 1) 39		1 1:	c Ar	g Se	r Le 39		n Fr	o As	rı Ar	9 Val 400
5	T'n	r Ph	e Ly:	s Al	a Ası 40:		g Pr	o Ph	ė Le	υ Va 41		÷ 11	e Ar	à ej	u Va 41	l Pro 5
	Lei	u Ası	n Th	r Il. 420		e Ph	е Ме	t Gl	y Ar 42		1 Al	a As	n Pr	o Cy: 430		l Lys
16	(2)	INI	FOPNI	AT I OI	N FO	R SE(	9 10	NO:	48:							
75			(	5EQU (A) I (B) I (D) I	ENGT YPE:	TH: 4	64 .no	amin acid								
73		(ii	.) MC	LECU	LE T	YPE:	pr	otei	ח							
				QUEN												
20 .	Met -32		-30		Val	Ile	Gly	7 Thi -25		l Thi	: Se:	: G1)	/ Lys		י דַיּיִי	. Val
	Tyr	Leu -15		Ser	Leu	Leu	Le: -10		e CJ?	, Phe	Trp	Asp -5	-	val	Thr	Cys
25	His l	_	Ser	Prc	Val 5		Ile	€ C7.5	Thr	Ala 10	-	Pro	Arç	Asp.	Ile 15	Pro
	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Arg	Ser 25		Glu	Lys	Lys	Ala 30	_	Glu
30	Asp	Glu	Gly 35	Ser	Glu	Gln	Lуs	11 ∈ 40		Glu	Ala	Thr	Asn 45	Arg	Arg	Val
	Trp	Glu 50	Leu	Ser	Ъуs	Ala	Asr. 55		Arg	Phe	Ala	Thr 60	Thr	Phe	Tyr	Gln
35	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	M∈t	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
. 40	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	IJ€	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125	Leu	Asn	Cys
45	Gln	Leu 130	Tyr	Gln	Asn	Ala	Asn 135	ГÀЗ	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	.Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
50	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
	Glu	As n	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn

	Ly	's <b>T</b> h	r Glu 195	Gly	' Arg	Ile	e Thi	: Asi 20(	o Va.	1 11:	e Pro	Se:	Gl: 209		a Ile	e Asn
5	Gl	u Lei 210	ı Thr	Val	Leu	Val	Leu 215	Va]	Ası	n Thi	: Ile	Ty:	Phe	≥ Lys	s Gly	/ Leu
	T: 22	p Lys 5	s Ser	Lys	Phe	Ser 230	Pro	Gli	ı Ası	n Thi	235	Fys	Glı	: Let	Phe	Tyr 240
10	Ly	s Ala	a Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	3 Ser 250		Met	Туг	: Gln	Glu 255	
	Ly	s Phe	e Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	ı Gly	Thr	Gln	Val	Leu 270		Leu
15	Pr	o Phe	275	Gly	Asp	Asp	Ile	Thr 250	Met	. Val	Leu	Ile	Leu 285		Lys	Pro
	Gl	1 Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
20	G1: 30:	n Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
25	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	) Met	Gly	Leu 340	Val	Asp	Leu	Phe	S∈r 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
30	Gly	'Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
35	Ser 385	Thr	Ala	Val	Val	Ala 390	Leu	GJ À	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys	Ala .	Asn . 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
40	Leu	Asn	Thr	Ile : 420	Ile	Phe	Met	Gly	Arg 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys

*0* 

	(2) INFORMATION FOR SEQ ID NO: 49:	
5	<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 24 base pairs</li><li>(B) TYFE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>	
	(ii) MOLECULE TYPE: DNA (genomic)	
10		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:	
	GTTTAGCGAC CGCGGAGCAA TCAC .	24
15	(2) INFORMATION FOR SEQ ID NO: 50:	
	<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 33 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li></ul>	
20	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:	
	GGGGTTTAGC GACCGCGGAA AAATCACAAC AGC	33
	(2) INFORMATION FOR SEQ ID NO: 51:	
30	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 30 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	-
	(ii) MOLECULE TYPE: DNA (genomic)	
35		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:	
	TAGCGAACGG CCGACAGCCA CAACAGCGGT	30
40	(2) INFORMATION FOR SEQ ID NO: 52:	
45	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:	
	CAGCGGTACT GCCAGCTGCT TC	22

	(2) INFORMATION FOR SEQ ID NO: 53:	
5	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 27 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
	(ii) MOLECULE TYPE: DNA (genomic)	
10		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:	
	ACGGCCAGCA ATCGGAACAG CGGTACT	27
15	(2) INFORMATION FOR SEQ ID NO: 54:	
20	<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 23 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>	
	(ii) MOLECULE TYPE: DNA (genomic)	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:	
	AATCACAACA AAGGTACTTG CAG	23
	(2) INFORMATION FOR SEQ ID NO: 55:	
30	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 30 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
35	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:	
40	GTTTAGCGAA CGCGGAATAA TCACAACAGC	30
	(2) INFORMATION FOR SEQ ID NO: 56:	
45	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 29 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
5 <i>0</i>		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:	

114

	GTTTAGCGAA CGCGGACCAA TCACAACAC	29
	(2) INFORMATION FOR SEQ ID NO: 57:	
5	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 30 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
10	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:	
	GTTTAGCGAA CGCGGATAAA TCACAACAGC	30
15	(2) INFORMATION FOR SEQ ID NO: 58:	
20	(i) SEQUENCE CHAFACTERISTICS:  (A) LENGTH: 30 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:	
	GTTTAGCGAA CGCGGCCAAA TCACAACAGC	30
	(2) INFORMATION FOR SEQ ID NO: 59:	
30	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 29 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
35	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:	
40	GTTTAGCGAA CGCGGAACAA TCACAACAG	29
	(2) INFORMATION FOR SEQ ID NO: 60:	
<i>4</i> 5	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 30 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
	(ii) MOLECULE TYPE: DNA (genomic)	
50		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:	

115

	TAGCGAACGG CCAATAGCCA CAACAGCGGT	30
	(2) INFORMATION FOR SEQ ID NO: 61:	
5	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 30 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
10	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:	
15	TAGCGAACGG CCAAGAGCCA CAACAGCGGT	30
	(2) INFORMATION FOR SEQ ID NO: 62:	
20	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 29 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
25	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:	
30	TAGCGAACGG CCAAGACCCA CAACAGCGG	29
	(2) INFORMATION FOR SEQ ID NO: 63:	
35	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 34 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
	(ii) MOLECULE TYPE: DNA (genomic)	
40		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:	
	GTTTAGCGAA CGGGGAACAG CCACAACAGC GGTA	34
45	(2) INFORMATION FOR SEQ ID NO: 64:	
50	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 34 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> <li>(ii) MOLECULE TYPE: DNA (genomic)</li> </ul>	

	(Mi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:	
	GTTTAGCGAA CGGGGAAAAA GCACAACAGC GGTA	3 4
5	(2) INFORMATION FOR SEQ ID NC: 65:	
10	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 30 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPGLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:	
	GTTTAGCGAA CGCGGAAGAA TCACAACAGC	3.0
	(2) INFORMATION FOR SEQ ID NO: 66:	
20	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 34 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
25	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:	
30	GTTTAGCGAA CGCGGATAAG CCACAACAGC GGTA	3 4
	(2) INFORMATION FOR SEQ ID NO: 67:	
35	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 33 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
40		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:	
	GTTTAGCGAA CGCGGCCAAG CCACAACAGC GGT	33
45	(2) INFORMATION FOR SEQ ID NO: 68:	
50	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 33 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
	(S) MOLECULE TYPE: DNA (denomic)	

. 55

	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:	
	GTTTAGCGAA CGCGGCCAAA GCACAACCGA GGT	33
5	(2) INFORMATION FOR SEQ ID NO: 69:	
10	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 29 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MCLECULE TYPE: DNA (genomic)	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:	
	GAAAGTCACC CTCTCGGGGT TTAGCGAAC	29
	(2) INFORMATION FOR SEQ ID NO: 70:	
20	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 32 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
25	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:	
30	TTGAAAGTCA CCCTCCTCGG GTTTAGCGAA CG	32
	(2) INFORMATION FOR SEQ ID NO: 71:	
35	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 32 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
40		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:	
	TTGAAAGTCA CCCGTCGACG GTTTAGCGAA CG	32
45	(2) INFORMATION FOR SEQ ID NO: 72:	
50	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 27 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	

	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:	
	CGGCAGTTCA GTTGGGCAAA GAAGAAG	2
5	(2) INFORMATION FOR SEQ ID NO: 73:	
70	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 30 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
	(ii) MOLECULE TYPE: DNA (genomic) .	
<b>7</b> 5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:	
	GGATTTGTTG GCGTTTTGAT AGAGTCGGCA	30
20	(2) INFOPMATION FOR SEQ ID NO: 74:	
	<ul> <li>(i) SEQUENCE CHAPACTERISTICS:</li> <li>(A) LENGTH: 19 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
25	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:	
30	GATAGAGTTG GCAGTTCAG	19
	(2) INFOPMATION FOR SEQ ID NO: 75:	1.0
35	(i) SEQUENCE CHAPACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STPANDEDNESS: single  (D) TOPOLOGY: linear	
40	(ii) MOLECULE TYPE: DNA (genomic) .	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:	
<i>4</i> 5.	GGTGGCCTCC AGGATCTTCT G	21
	(2) INFORMATION FOR SEQ ID NO: 76:	
50	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 39 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	

	(ii) MOLECULE TYPE: DNA (genomic)	
_	(with COUNTY PRODUCTION AND TO US	
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:	
	GGGATTCATG GGAATGGATC GTGGGATTGC TGTGCAGAT	39
	(2) INFORMATION FOR SEQ ID NO: 77:	
10	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 21 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
15	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:	
20	GTTGGCTTTT TGATAGAGTC G	21
	(2) INFORMATION FOR SEQ ID NO: 78:	
25	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:	
	TTTGTTGGCG TTTCGATAGA G	21
	(2) INFORMATION FOR SEQ ID NO: 79:	
35	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
40	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:	
45	TTTGTTGGCT TGTCGATAGA G	21
	(2) INFORMATION FOR SEQ ID NO: 80:	
50	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 25 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	

	(ii) MOLECULE TYPE: DNA (genomic)	
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80: TACATGGCCG AAGCTTEGTA ATCAT	25
10	(2) INFORMATION FOR SEQ ID NO: 81:  (i) SEQUENCE CHAPACTERISTICS:  (A) LENGTH: 29 base pairs  (B) TYPE: nucleic acid	
15	(C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (genomic)	
20	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81: CAAAGAATAA GATCTTATTA CTTAACACA	29
25	Claims	
30	1. A human antithrombin III (AT III) mutant obtained by subjecting human AT III to mutation, which human AT III amino acid sequence described below except that an amino acid(s) mutates into an amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-positions, the 125- to 133-positions and the 384- to 398-positions:	nothe
35		
40		
<b>4</b> 5		
50		
55		

# human AT III amino acid sequence

5																	
		Met	Tyr	Ser	Asn	Val	lle	Gly	Thr	Val	Thr	Ser	Gly	Lys	Arg	Lys	Val
		-32		-30					-25					-20			
10		Tyr	Leu	Leu	Ser	Leu	Leu	Leu	lle	Gly	Phe	Trp	Asp	Cys	Val	Thr	Cys
			-15					-10					-5				
		His	Gly	Ser	Pro	Yal	Asp	lle	Cys	Thr	Ala	Lys	Pro	Arg	Asp	lle	Рго
15		1				5					10					15	
		Met	Asn	Pro	Met	Cys	lle	Tyr	Arg	Ser	Pro	Glu	Lys	Lys	Ala	Thr	Glu
					20					25					30		
20		Asp	Glu	Gly	Ser	Glu	Gln	Lys	lle	Pro	Glu	Ala	Thr	Asn	Arg	Arg	Val
				35					40					45			
	·	Trp		Leu	Ser	Lys	Ala	Àsn	Ser	Arg	Phe	Ala	Thr	Thr	Phe	Tyr	Gln
25			50					5 5					60				
		His	Leu	Ala	Asp	Ser	Lys	Asn	Asp	Asn	Лsр	Asn	lle	Phe	Leu	Ser	Pro
		6 5					70					75					80
30		Leu	Ser	He	Ser		Ala	Phe	Ala	Met	Thr	Lys	Leu	Gly	Лlа	Cys	Asn
						85					90					95	
35																	
40																	
45																	
40																	
50																	
50																	

	As	p Thi	r Lei	u Glr	Gli	n Lei	и Ме	i Gl	u Va	l Ph	e Ly	s Ph	e As	p Th	r 11	e Sei
				100	)				10	5				11	0	
5	Gla	ı Lys	Th a	Ser	Vel	o Gli	1110	e llis	5 Phe	= Pho	≥ Ph	e Al	a <u>Ly</u>	s Le	u Ası	n Cys
			115	)				120	)				12	5		
	Arg	Leu	Tyr	Arg	Lys	s Ala	a Asr	n Lys	Sei	Sei	Ly:	s Le	u Va	l Se	r Ala	Asr
70		130	)				135	)				140	0			
	Arg	Leu	Phe	Gly	Asp	Lys	Ser	Leu	Thr	Phe	e Ası	ı Gli	u Thi	Ty:	r Glr	Asp
	145	ı				150	ı				155	5				160
75	Ιle	Ser	Glu	Leu	Va l	Tyr	Gly	Ala	Lys	Leu	Glr	Pro	Lei	ı Ası	? Phe	Lys
					165					170					175	
	Glu	Asn	Ala	Glu	Gln	Ser	Arg	Ala	Ala	lle	Asn	Lys	Trp	Val	Ser	Asn
20				180					185					190	)	
.•	Lys	Thr	Glu	Gly	Arg	lle	Thr	Asp	Val	He	Pro	Ser	Glu	Ala	lle	Asn
			195					200					205			•
N.T.	Glu	Leu	Thr	Val	Leu	Val	Leu	Val	Asn	Thr	lle	Tyr	Phe	Lys	Gly	Leu
25		210					215					220				
	Trp	Lys	Ser	Lys	Phe	Ser	Pro	Glu	Asn	Thr	Arg	Lys	Glu	Leu	Phe	Tyr
	225					230					235					240
0	Lys	Ala	Asp	Gly		Ser	Cys	Ser	Ala	Ser	Met	Met	Туг	Gln	Glu	Gly
					245					250					255	
	Lys	Phe	Arg	Туг	Arg	Arg	Val	Ala	Glu	Gly	Thr	Gln	Val	Leu	Glu	Leu
5				260					265					270		
	Рго	Phe	Lys	Gly	Asp	Asp	lle	Thr	Меt	Val	Leu	He	Leu	Pro	Lys	Рто
			275					280					285			
0	Glu	Lys	Ser	Leu	Ala	Lys	Val	Glu	Lys	Glu	Leu	Thr	Pro	Glu	Val	Leu
		290					295					300				
	Gln	Glu	Trp	Leu	Asp	G] u	Leu	Glu	Glu	Met	Met	Leu	Val	Vаl	His	Met
5	305					310					315					320
	Pro	Arg	Phe	Arg	lle	Glu	Asp	Gly	Phe	Ser	Leu	Lys	Glu	Gln	Leu	Gln
					325					330					335	

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Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala Ser Thr Ala Val Val Ile Ala Gly Arg Ser Leu Asn Pro Asn Arg Val Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 

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2. The human AT III mutant as claimed in Claim 1, wherein said another amino acid(s) is selected from the group consisting of Ala, Gly, Trp, Pro, Leu, Val, Phe, Tyr, Ile, Glu, Ser, Gln, Asn and Arg.

3. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions, the 41- to 47-positions and the 125- to 133-positions.

4. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions and the 41- to 47-positions.

5. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions and the 125- to 133-positions.

6. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 41-to 47-positions and the 125- to 133-positions.

7. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 11- to 14-positions and that an amino acid(s) mutates into another amino acid(s) at the 384-to 398-positions.

50 8. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 41- to 47-positions and that an amino acid(s) mutates into another amino acid(s) at the 384-to 398-positions.

9. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 125- to 133-positions and that an amino acid(s) mutates into another amino acid(s) at the 384-to 398-positions.

- 10. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- 11. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) at the 384- to 398-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val, Gly, Arg, Glu and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
- 12. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) at the 390- to 392-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
  - 13. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
  - 14. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of IIe at the 390- position into AIa, a mutation of AIa at the 391- position into Phe, Val or Leu and a mutation of GIy at the 392-position into Pro is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
  - 15. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Ala at the 384- position into Gly, a mutation of Ala at the 387- position into Phe, a mutation of Val at the 389-position into Pro, a mutation of Pro at the 397- position into Arg and a mutation of Asn at the 398-position into Glu or Alg is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
- 16. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into IIe, a mutation of Asp at the 14- position into Ser is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions, the 125-to 133-positions and the 384- to 398-positions.
  - 17. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into IIe and a mutation of Asp at the 14- position into Ser, and, another mutation selected from the group consisting of a mutation of IIe at the 390- position into Ala, a mutation of Ala at the 391- position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions and the 125- to 133-positions.
  - 18. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 125- position into Gln, a mutation of Arg at the 129- position into Gln, a mutation of Arg at the 132-position into Gln and a mutation of Lys at the 133- position into Asn or Gln is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 384- to 398-positions.
  - 19. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 125- position into Gln, a mutation of Arg at the 129- position into Gln, a mutation of Arg at the 132-position into Gln and a

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mutation of Lys at the 133- position into Asn or Gln, and, another mutation selected from the group consisting of a mutation of Ile at the 390- position into Ala, a mutation of Ala at the 391- position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions and the 41- to 47-positions.

- 20. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro.
- 21. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
  - 22. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that IIe-Ala at the 390- to 391-positions mutates into Ala-Leu.
  - 23. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Lys at the 125-position mutates into Gln and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- 24. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and Ile-Ala at the 390- to 391-positions mutates into Ala-Leu.
- 25. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- 26. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Lys at the 133-position mutates into Asn and Ala-Gly at the 391- to 392-positions mutates into Phe30
  Pro.
  - 27. A DNA coding for the human AT III mutant as claimed in Claim 1.
- 28. An expressible vector which has a DNA containing part or the whole of the DNA sequence coding for the human AT III mutant as claimed in Claim 1.
  - 29. A transformant which is obtained by subjecting host cells to transformation with the expressible vector as claimed in Claim 28.
- 40 30. The transformant as claimed in Claim 29, wherein the host cells are Escherichia coli or animal cells.
  - 31. A method for producing a human AT III mutant which comprises incubating the transformant as claimed in Claim 30 and recovering the human AT III mutant produced by the transformant from the culture.
- 32. A drug composition for thrombotic disorders which contains the human AT III mutant as claimed in Claim 1 and pharmaceutically acceptable carriers.
  - 33. A use of the human AT III mutant as claimed in Claim 1 for the making of a medicament for treating thrombotic disorders.

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Fig. 1

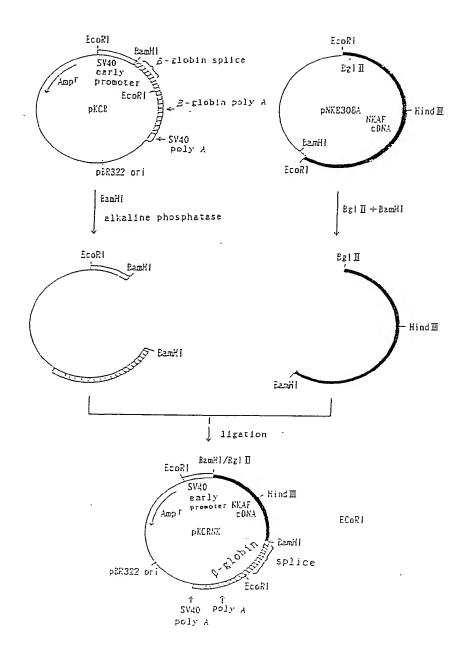


Fig. 2

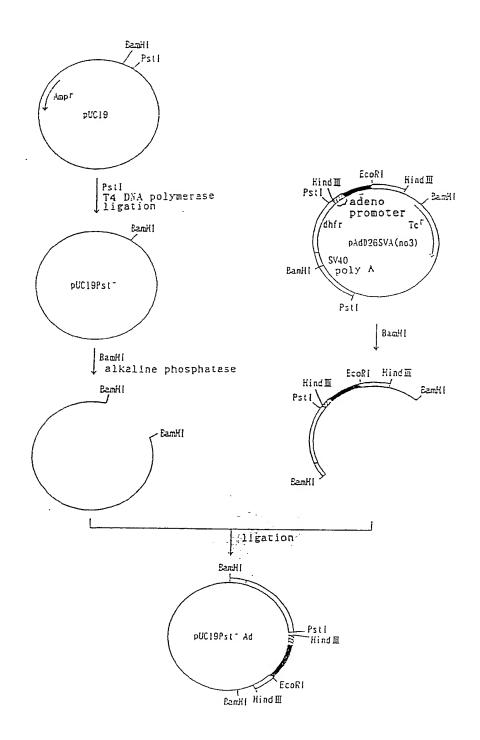


Fig. 3

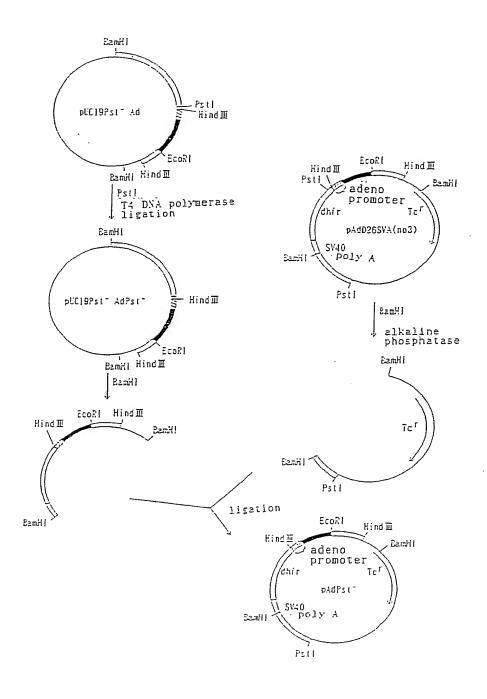


Fig. 4

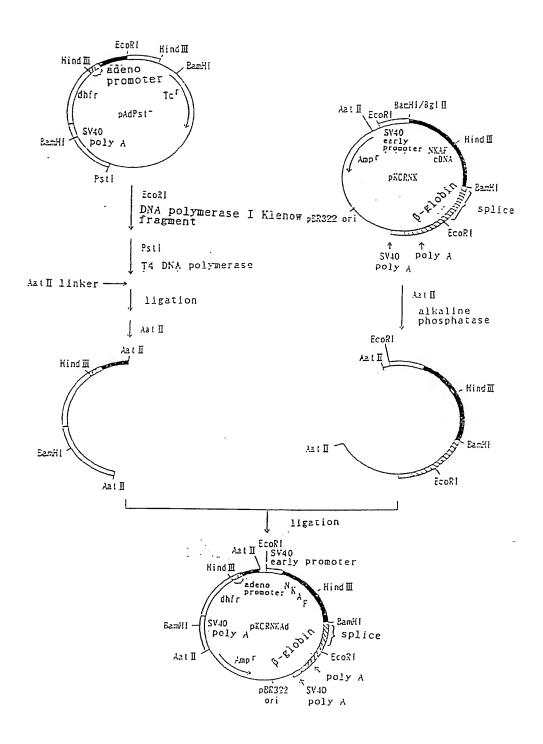


Fig. 5

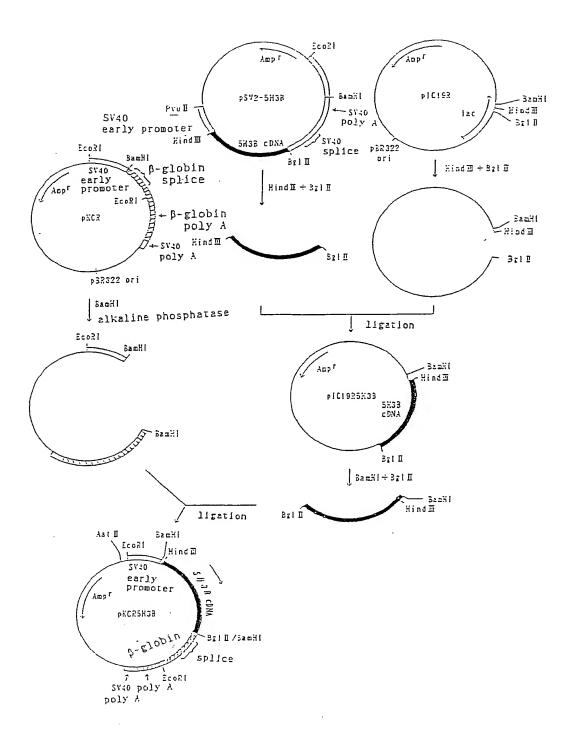


Fig. 6

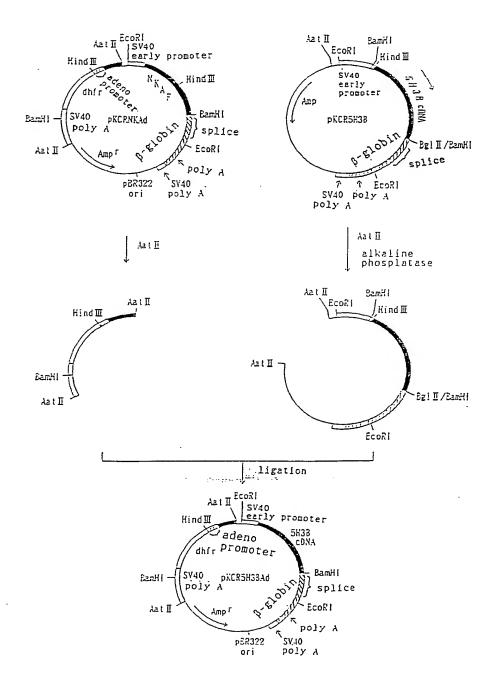
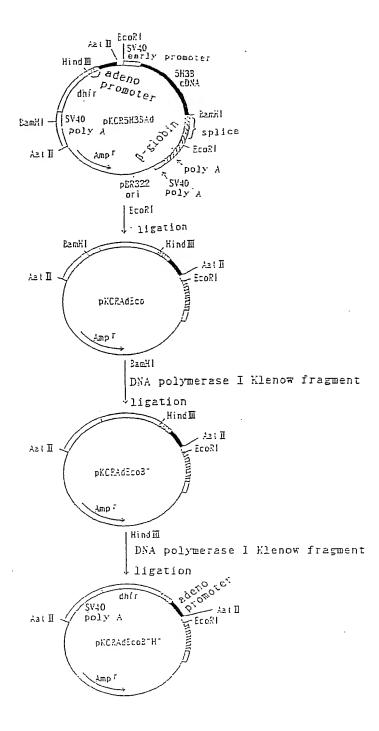


Fig. 7



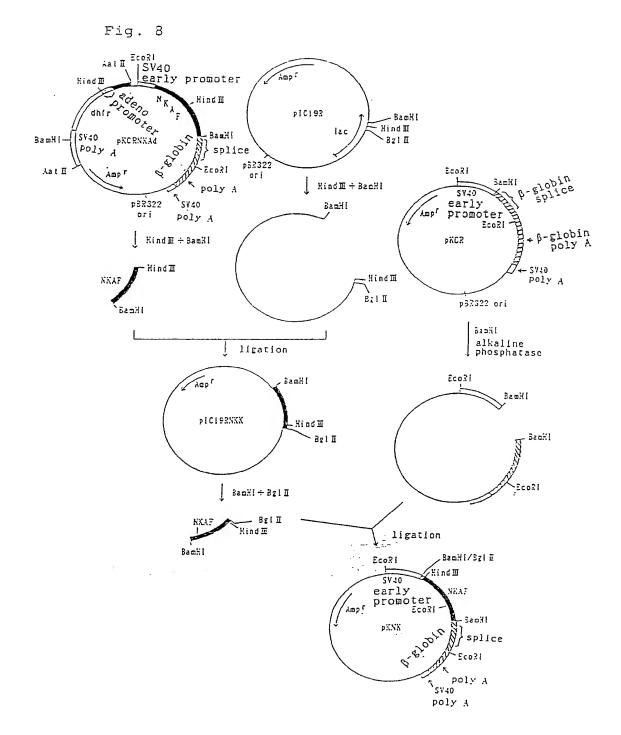


Fig. 9

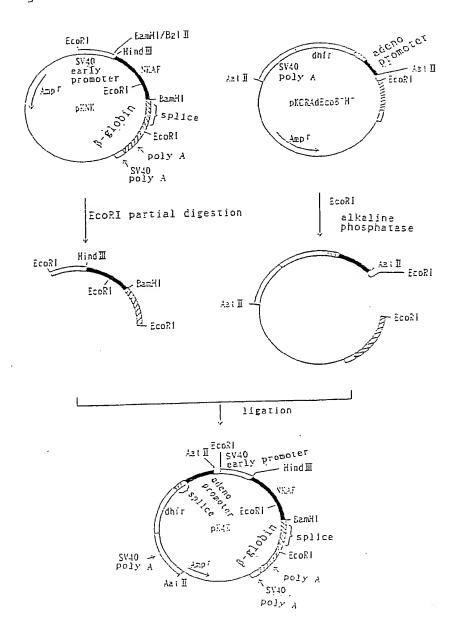
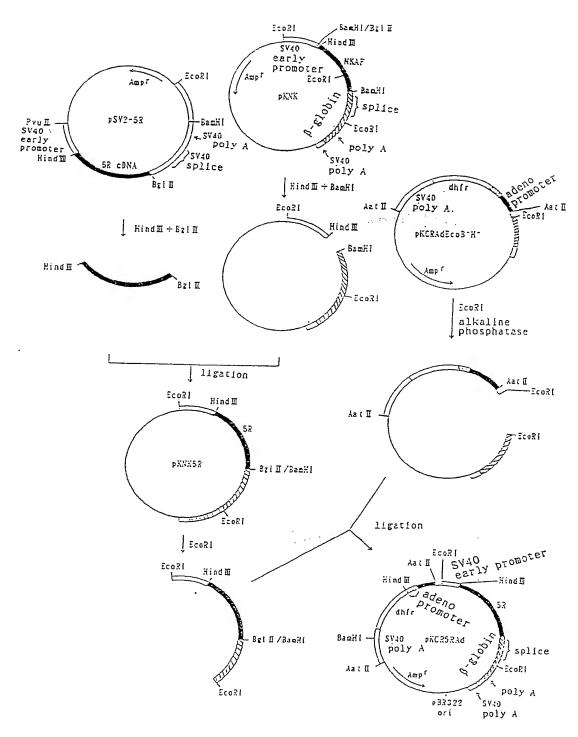


Fig. 10





#### EUROPEAN SEARCH REPORT

Application Number

EP 93 10 5829

_	DOCUMENTS CONSIDE					
Category	Citation of document with indica of relevant passag	ation, where appropriate, es	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Ct.5)		
X,C	WO-A-9 100 291 (AKZO N		1,2, 27-33	C07K15/00 A61K37/64 C12N15/15		
o,x	EP-A-0 384 122 (BEHRIN		1,2,			
	* the whole document *	•	27-33			
١	EP-A-0 424 351 (WASHIN * abstract; claims 1-1	GTON UNIVERSITY)	1,27-33			
		- <b></b>				
				TECHNICAL FIELDS SEARCHED (Int. Cl.5)		
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	The present search report has been d	trawn up for all claims				
	Place of search	Date of completion of the search		Examiner		
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